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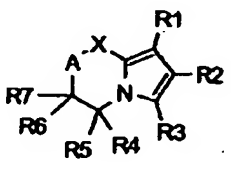

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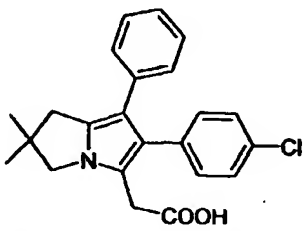

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(57) Abstract: Inhibiting gastric proton pump in a mammal is accomplished by the use of a compound of formula (1) wherein the variables have the meaning given in the present description. A preferred compound of formula (2) is this treatment ameliorates, diminishes, actively treats, reverses or prevents any injury, damage or lesions of gastric mucosa, e.g. gastric mucosal lesions and ulceration.

WO 03/097041 A1

ANNELLATED PYRROLE COMPOUNDS AS PROTON PUMP INHIBITORS FOR TREATING ULCER

The present invention relates to the use of annellated pyrrole compounds and in particular ML3000, salts or derivatives thereof, as proton pump inhibitors.

BACKGROUND OF THE INVENTION

Peptic ulcers are one of the most prevalent diseases in industrialized nations.

Acid secretory inhibitors are among the most highly-prescribed medications, reflecting the physiological adage "No acid, no ulcer". Pharmacological or pathophysiological breaching of the gastric mucosal barriers to back diffusion of HCl leads to rapid erosion of the gastric epithelial monolayer and consequent ulceration of the mucosa. Inhibition of acid secretion by antagonism at the parietal cell histamine H_2 receptor (cimetidine), or by direct covalent derivatization and inactivation of the gastric proton pump (omeprazole, lansoprazole, rabeprazole) is routine for amelioration and promotion of healing of gastric ulcers. The gastric proton pump is an enzyme which is also known as H^+/K^+-ATPase. It is located in the membrane of gastric parietal cells and is responsible for the transport of protons from blood to lumen, which in turn results in decreasing the pH of stomach contents.

Omeprazole itself is in fact a prodrug which under acidic conditions converts to the active drug, namely its corresponding sulfenamide. The mechanism of action of omeprazole is well-studied and is known to involve a nucleophilic attack of one (or two) thiol group(s) of the H^+/K^+-ATPase on the sulfur atom of the chemically active sulfenamide. The resulting chemical modification of the thiol group(s) of the enzyme (formation of a disulfide bond between the H^+/K^+-ATPase sulfur and the sulfur of the benzimidazole pyridinium salt) causes the observed inhibition of the proton pump. It should be emphasized however, that the conversion of the prodrug to the active enzyme inhibitor can only be achieved in acidic media which also results in substantial degradation of the active sulfenamide. In summary, the instability of omeprazole in acidic environments, which is a prerequisite to its activation into a proton pump inhibitor, is the major shortcoming of this drug.

In addition to acid secretion interleukin-8 secretion represents a further gastric mucosal function which plays an important role in gastric ulceration.

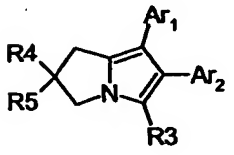
The ulcerogenic bacterium Helicobacter pylori has been shown to increase the rate and amplitude of IL-8 secretion both in vitro and in vivo, and to up-regulate IL-8 gene expression. Similar IL-8 prosecretory effects are seen as a result of IL-1b stimulation of gastric epithelial cells.

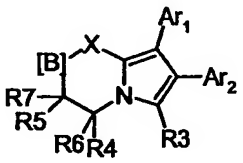
Nonsteroidal antiphlogistika (NSAIDs), such as acetylsalicylic acid (ASA), diclofenac, indomethacin, ibuprofen and naproxen, are widely used in the clinic. From a pharmacological point of view they act as inhibitors of the cyclooxygenase (COX).

The anti-inflammatory properties of NSAIDs are related to their suppression of prostaglandin synthesis. However, suppression of gastric prostaglandins decreases gastric mucosal blood flow, with concomitant mucosal sensitivity to topical injury by a variety of irritants. Gastric ulceration induced by NSAIDs significantly limits the utility of these drugs.

Pyrrolizines which pharmacologically act similar, are known from numerous publications. For instance, antiphlogistically active pyrrolizines are described in Arch. Pharm. 319, 65-69 (1986); 319, 231-234 (1986); 318, 661-663 (1985); 318, 663-664 (1985); 319, 500-505 (1986); 319, 749-755 (1986); 327, 509-514 (1994); 330, 307-312 (1997) as well as in J. Med. Chem. 1987, 30, 820-823 and 1994, 37, 1894-1897.

Further pyrrolizines can be taken from US 5,260,451 (corresponding to EP 0397175) as well as from WO 95/32970; WO 95/32971; and WO 95/32972. These compounds are represented by the structural formula

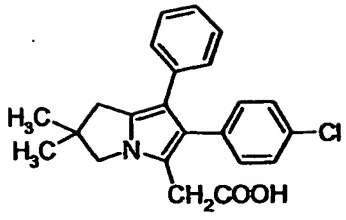




and share an annellated diarylpyrrol moiety as well as a third acidic residue R3. The compounds are characterized by a high lipophilicity, good bioavailability and half-lifes in the medium range, s. Drugs of the Future, 1995, 20 (10):1007-1009.

Further pyrrolizines of similar constitution are described in DE 198 45 446.6 and WO 01/05792. Moreover, alkylsulfinylbenzoyl and alkylsulfonylbenzoyl substituted pyrrolizines, according to US 4,232,038, are said to have anti-inflammatory, analgetic and antipyretic properties. According to DE 196 24 290.8 and DE 196 24 289.4 certain compounds of this type have a lipid-reducing action.

ML-3000 ([2,2-dimethyl-6-(4-chlorophenyl)-7-phenyl-2,3-dihydro-1H-pyrrolizine-5 yl]-acetic acid) of the Formula (Ia)

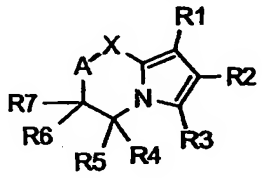


is a non-antioxidant balanced dual inhibitor of COX and 5-Lipoxygenases (5-LO) (1). The drug is a nonselective inhibitor of COX, inhibiting both COX-1 and COX-2. This drug has analgesic, antipyretic and anti-inflammatory activity, and has been demonstrated to have potent anti-inflammatory action in a number of animal models including carrageenan-induced paw edema in the rat, and rat adjuvant arthritis (2). Further, it has been reported that ML3000 shows excellent gastrointestinal tolerance (12, 13). Gastoprotective properties, however, have not been observed (11).

Surprisingly, it has been found that certain annellated pyrrole compounds, such as ML-3000, have a significant gastroprotective effect. This phenomenon is associated not only with a significant inhibition of the gastric proton pump, but also with the inhibition of IL-8 secretion in gastric epithelial cells.

SUMMARY OF THE INVENTION

Thus, the present invention relates to the use of annellated pyrrole compounds represented by the general formula (I):



wherein

X represents

CR8R9, S, O, NR12 or C(O);

A represents

CR10R11 or a bond between X and the atom carrying radicals R6 and R7;

the first of radicals R1, R2, R3 represents

aryl, optionally substituted with one or more than one substituents independently selected among the group consisting of halogen, alkyl, halogenoalkyl, alkoxy, aryloxy, halogenoalkoxy, alkylthio, hydroxy, nitro, alkylsulfinyl, alkylsulfonyl, sulfamoyl, N-alkylsulfamoyl, N,N-di-alkylsulfamoyl, alkylsulfonamido and alkylsulfon-N-alkylamido; or

an aromatic or non-aromatic, mono- or bicyclic, optionally benzoannellated, heterocyclic group having 1, 2 or 3 heteroatoms independently selected from N, O and S and optionally being substituted with one or more than one substituents independently selected among the group consisting of halogen, alkyl, halogenoalkyl, alkoxy, aryloxy, halogenoalkoxy, alkylthio, hydroxy, nitro, alkylsulfinyl, alkylsulfonyl, sulfamoyl, N-alkylsulfamoyl, N,N-di-alkylsulfamoyl, alkylsulfonamido and alkylsulfon-N-alkylamido;

the second of radicals R1, R2, R3 represents

alkyl, optionally substituted with one or more than one substituents independently selected among the group consisting of halogen, cycloalkyl, alkoxy, trifluormethoxy, hydroxy and trifluormethyl;

cycloalkyl, optionally substituted with one or more than one substituents independently selected among the group consisting of halogen, alkyl, halogenoalkyl, cycloalkyl, alkoxy, halogenalkoxy and hydroxy;

aryl, optionally substituted with one or more than one substituents independently selected among the group consisting of halogen, alkyl, halogenoalkyl, alkoxy, aryloxy, halogenoalkoxy, alkylthio, hydroxy, nitro, alkylsulfinyl, alkylsulfonyl, sulfamoyl, N-alkylsulfamoyl, N,N-di-alkylsulfamoyl, alkylsulfonamido and alkylsulfon-N-alkylamido; or

an aromatic or non-aromatic, mono- or bicyclic, optionally benzoannellated, heterocyclic group having 1, 2 or 3, heteroatoms independently selected from N, O and S and optionally being substituted with one or more than one substituents independently selected among the group consisting of halogen, alkyl, halogenoalkyl, alkoxy, aryloxy, halogenoalkoxy, alkylthio, hydroxy, nitro, alkylsulfinyl, alkylsulfonyl, sulfamoyl, N-alkylsulfamoyl, N,N-di-alkylsulfamoyl, alkylsulfonamido and alkylsulfon-N-alkylamido;

the third of radicals R1, R2, R3 represents

H, alkyl, halogenoalkyl, hydroxyalkyl, -CHO, -COOH, halogen, cyano, alkylsulfonyl, sulfamoyl or B-Y;

wherein

B represents alkylene or alkenylene, optionally substituted with hydroxy or alkoxy;

Y represents -COOH, SO_3H, $OPO(OH)_2$, $OP(OH)_2$, -CHO or tetrazolyl; or

the second and the third of radicals R1, R2, R3 represent,

together with the atom they are attached to, saturated or unsaturated cycloalkyl;

R4-R11, which may be the same or different, represent

hydrogen, alkyl, hydroxyalkyl, alkoxyalkyl, hydroxy, COOH or acyloxy, where vicinal radicals may also represent bonds or geminal radicals, together with the C atom they are attached to, may also represent carbonyl or cycloalkyl;

R12 represents

hydrogen, alkyl or phenyl,

and optical isomers, physiologically acceptable salts and derivatives thereof,

for inhibiting gastric proton pump.

The term "alkyl, alkoxy etc." includes linear or branched alkyl groups, such as CH_3, C_2H_5, n-propyl, $CH(CH_3)_2$, n-butyl, $CH(CH_3)$-C_2H_5, isobutyl, $C(CH_3)_3$, n-pentyl or n-hexyl, in particular CH_3, C_2H_5 or $CH(CH_3)_2$, preferably having – unless otherwise stated - 1 to 8, in particular 1 to 6 and more preferably 1 to 4 carbon atoms; as a substituent of a radical R1 to R12 „alkyl, alkoxy etc." preferably comprises 1 to 4 carbon atoms.

Substituted „alkyl, alkoxy etc." includes in particular:

halogenoalkyl, i.e., alkyl, which is partially or completely substituted with fluoro, chloro, bromo and/or iodo, e.g. CH_2F, CHF_2, CF_3, CH_2Cl, 2-fluoroethyl, 2-chloroethyl or

2,2,2-trifluoroethyl; as a substituent of a radical R1 to R12 halogenoalkyl preferably means CHF_2 and especially CF_3;

halogenoalkoxy, i.e., alkoxy, which is partially or completely substituted with fluoro, chloro, bromo and/or iodo, e.g. halogenoalkoxy residues corresponding to the afore-mentioned halogenoalkyl residues; as a substituent of a radical R1 to R12 halogenoalkoxy preferably means $OCHF_2$ and especially OCF_3;

alkoxyalkyl, i.e., alkyl substituted by alkoxy, e.g. $-CH_2-OCH_3$ or 2-Methoxyethyl;

hydroxyalkyl, i.e., alkyl which is – preferably mono – substituted by hydroxy, e.g., hydroxymethyl or 2-hydroxyethyl;

trifluoromethylalkyl, i.e. alkyl, which is – preferably mono – substituted by trifluoromethyl, e.g., the residues as described in respect of hydroxyalkyl which are substituted with trifluormethyl instead of hydroxy;

trifluoromethoxyalkyl, i.e. alkyl, which is – preferably mono – substituted by trifluoromethoxy, e.g., the residues as described in respect of hydroxyalkyl which are substituted with trifluormethxy instead of hydroxy;

cycloalkylalkyl, i.e., alkyl, which is – preferably mono – substituted by cycloalkyl, e.g. the residues as described in respect of hydroxyalkyl which are substituted with cyclopropyl, cyclobutyl, cyclopentyl or cyclohexyl instead of hydroxy.

The term "cycloalkyl" includes mono- or bicyclic alkyl groups, such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, etc., preferably having – unless otherwise stated - 3 to 9, in particular 3 to 7 and more preferably 5 or 6 carbon atoms.

The term „alkylene" includes linear or branched alkylene groups, such as methylene and ethylene, preferably having – unless otherwise stated - 1 to 8, in particular 1 to 6 and more preferably 1 to 4 carbon atoms. If alkylene is substituted with hydroxyl or alkoxy, monosubstitution is preferred.

The term „alkenylene" includes linear or branched, mono- or polyunsaturated alkylene groups, such as ethenylene, preferably having – unless otherwise stated - 2 to 8, in particular 2 to 6 and more preferably 2 to 4 carbon atoms. If alkenylene is substituted with hydroxyl or alkoxy, monosubstitution is preferred.

Acyloxy means –OCOR, wherein R represents alkyl or aryl. Preferred examples are acetyloxy and benzoyloxy.

-COOAlkyl means alkoxycarbonyl, such as $CO-OCH_3$, $CO-OC_2H_5$, $CO-OCH_2-C_2H_5$, $CO-OCH(CH_3)_2$, n-butoxycarbonyl, $CO-OCH(CH_3)-C_2H_5$, $CO-OCH_2-CH(CH_3)_2$, $CO-OC(CH_3)_3$, in particular $CO-OCH_3$, $CO-OC_2H_5$, $CO-OCH(CH_3)_2$ or $CO-OCH_2-CH(CH_3)_2$.

-COOAlkPhenyl means an alkoxycarbonyl group which is substituted on the alkyl moiety with phenyl, such as benzyloxycarbonyl.

Alkylthio means –S-Alkyl and is also referred to as alkylsulfanyl or alkylmercapto, such as SCH_3, SC_2H_5, $SCH_2-C_2H_5$, $SCH(CH_3)_2$, n-butylthio, 1-methylpropylthio, 2-methylpropylthio, $SC(CH_3)_3$. Methylthio is preferred.

Alkylsulfinyl means –S(O)-Alkyl and is also referred to as alkylsulfoxo, such as $SO-CH_3$, $SO-C_2H_5$, n-propylsulfinyl, 1-methylethylsulfinyl, n-butylsulfinyl, 1-methylpropylsulfinyl, 2-methylpropylsulfinyl, 1,1-dimethylethylsulfinyl. Methylsulfinyl is preferred.

Alkylsulfonyl means $-S(O)_2$-Alkyl and is also referred to as alkylsulfone, such as SO_2-CH_3, $SO_2-C_2H_5$, n-propylsulfonyl, $SO_2-CH(CH_3)_2$, n-butylsulfonyl, 1-methylpropylsulfonyl, 2-methylpropylsulfonyl, $SO_2-C(CH_3)_3$. Methylsulfonyl is preferred.

Sulfamoyl means $-S(O)_2NH_2$ and is also referred to as amidosulfonyl or sulfonic acid amid.

N-Alkylsulfamoyl means mono-substituted sulfamoyl $-S(O)_2NH$-Alkyl, e.g. $-S(O)_2NH-CH_3$.

N,N-Dialkylsulfamoyl means di-substituted sulfamoyl $-S(O)_2N-(Alkyl)_2$, wherein the N-bounded alkyl residues may be the same or different, e.g. $-S(O)_2N(CH_3)_2$.

Alkylsulfonamido means $-NHS(O)_2$-Alkyl, such as $NHSO_2-CH_3$, $NHSO_2-C_2H_5$, n-propylsulfonamido, $NHSO_2-CH(CH_3)_2$, n-butylsulfonamido, 1-methylpropylsulfonamido, 2-methylpropylsulfonamido, $NHSO_2-C(CH_3)_3$. Methylsulfonamido is preferred.

Alkylsulfon-N-alkylamido means –N(Alkyl)$S(O)_2$-Alkyl, wherein the N- and the S-bounded alkyl residues may be the same or different, e.g $N(CH_3)SO_2-CH_3$.

Carbonyl, CHO, -COOH, $-SO_3H$ means >C=O, formyl, carboxy, carboxycarbonyl and sulfo, respectively.

"Aryl" preferably means naphthyl and in particular phenyl.

The term "halogen" includes a fluoro, chloro, bromo or iodo atom. Usually fluoro and chloro, and in some cases also bromo are preferred.

"Heterocyclic residues" include in particular 5- or 6-membered heterocyclic residues which may be aromatic or non-aromatic, mono- or bicyclic, and/or benzoannellated. Examples are nitrogen-containing heterocyclic residues, such as pyrrolyl, imidazolyl, pyrazolyl, pyridazinyl, pyrazinyl, indolyl, chinolinyl, especially pyridyl, pyrimidyl and isochinolinyl. The aromatic residues also include heterocyclic residues which contain an oxygen or a sulfur atom, such as thienyl, benzothienyl, furanyl and especially benzofuranyl. Also included are heterocyclic residues which contain 2 or more than 2 different heteroatoms, such as thiazolyl, isothiazolyl, thiadiazolyl, isoxazolyl and

oxazolyl. Thienyl, pyridyl and thiazolyl are preferred aromatic heterocyclic residues. Non-aromatic residues include nitrogen-containing heterocyclic residues, such as pyrrolidinyl, piperidinyl and piperazinyl. This also includes heterocyclic residues which contain 2 or more than 2 different heteroatoms, such as morpholinyl.

Substituted residues, in particular alkyl, cycloalkyl, aryl and heteroaryl, are preferably mono-, di- or tri-substituted.

The [α]-annelland may be 6- or especially 5-membered, heterocyclic or especially alicyclic, if alicyclic, then unsaturated or especially saturated, and/or substituted or unsubstituted.

The [α]-annellated pyrrole compounds of Formula (I) include in particular those wherein X represents CR8R9 and A represents a bond between X and the atom carrying radicals R6 and R7 (pyrrolizines); X represents CR8R9 and A represents CR10R11 (indolizines); X represents NR12 and A represents a bond between X and the atom carrying radicals R6 und R7 (pyrrolo[1,2-a]imidazoles); X represents S and A represents a bond between X and the atom carrying radicals R6 and R7 (pyrrolo[2,1-b]thiazoles); X represents S and A represents CR10R11 (pyrrolo[2,1-b]1,3-thiazines); X represents O and A represents CR10R11 (pyrrolo[2,1-b]1,3-oxazines); X represents O and A represents a bond between X and the atom carrying radicals R6 and R7 (pyrrolo[2,1-b]oxazoles), residues not mentioned having the meanings given above.

If the [α]-annelland is a 5-membered unsaturated residue, especially R4 and R6 represent a bond, such as, e.g., in pyrrolizine, pyrrolo[2,1-b]imidazole and pyrrolo[2,1-b]thiazole. If the [α]-annelland is a 6-membered unsaturated residue, especially R4 and R6, such as, e.g., in pyrrolo[2,1-b]1,3-thiazine, pyrrolo[2,1-b]1,3-oxazine or 5,6-dihydroindolizine, and optionally also R8 and R10, such as, e.g., in indolizine, represent a bond.

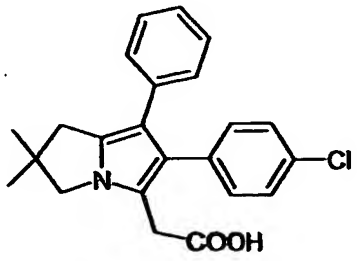
Without being bound to a specific [α]-annelland, according to a particular embodiment of the invention, R4-R11 which may be the same or different represent hydrogen or alkyl. According to a further particular embodiment of the invention, at least one of radicals R4, R5, R6 and R7 represents hydroxyalkyl, in particular hydroxymethyl, and the remaining radicals R4, R5, R6 and R7 independently represent H or alkyl. According to this embodiment it is preferred that R4 is hydroxyalkyl, in particular hydroxymethyl, and R5 is H or alkyl, and R6, R7 independently are H or alkyl. According to a further particular embodiment of the invention, one of radicals R8 and R9 represents H, alkyl, hydroxyalkyl or alkoxyalkyl and the other represents hydroxyl, alkoxy, carboxyl or acyloxy, or R8 and R9 together with the C atom they are attached to, represent a carbonyl group.

6,7-Dihydro-5H-pyrrolizines are especially useful, i.e. compunds of Formula (I), wherein X represents CR8R9, A represents a bond between X and the atom carrying radicals R6 und R7, and R4, R5, R6, R7, R8, R9 which may be the same or different, have the meaning as given above and preferably represent hydrogen or alkyl. 6,7-Dihydro-5H-pyrrolizine wherein R4 to R9 are hydrogen or at least one or two of radicals R4 to R9, for instance R6 und/oder R7, represent alkyl, in particular methyl, are especially preferred.

According to an important aspect of the present invention, compounds of Formula (I), wherein the first and the second of radicals R1, R2, R3, preferably R1 and R2, independently represent an Π-electon-rich system selected from aryl and aromatic heterocyclic residues, in particular phenyl, optionally substituted with one or more than one substituents that in particular are independently selected among the group consisting of halogen, alkyl and halogenoalkyl, in particular CF_3, R1 being preferably unsubstitued phenyl and R2 being preferably 4-substituted phenyl, are especially useful.

According to a further important aspect of the invention, compounds of Formula (I), wherein the third of radicals R1, R2, R3, preferably R3, represents an acidic residue such as COOH or B-Y, wherein Y is COOH and B preferably represents alkylene, or represents a precursor of an acidic residue such as B-Y, wherein Y is tetrazolyl, are especially useful.

The use of [6-(4-chlorophenyl)-2,2-dimethyl-7-phenyl-2,3-dihydro-1H-pyrrolizine-5-yl]-acetic acid (ML3000) represented by formula (Ia):



its physiologically acceptable salts and derivatives, e.g., physiologically hydrolysable esters, is especially preferred.

Physiologically acceptable salts include acid or base addition salts.

Acid addition salts are, for instance, salts of compounds of Formula (I) with inorganic acids, such as hydrochloric acid, sulfuric acid, nitric acid or phosphoric acid, or with organic acids, in particular carboxylic acids, e.g. acetic acid, tartaric acid, lactic acid, citric acid, malic acid, amygdalic acid, ascorbic acid, maleic acid, fumaric acid,

gluconic acid or sulfonic acid, e. g. methanosulfonic acid, phenylsulfonic acid and toluenesulfonic acid, and the like.

Base addition salts are, for instance, salts of compounds of Formula (I) with inorganic bases, such as sodium or potassium hydroxide or with organic bases, such as mono-, di- or triethanolamine, and the like.

Physiologically acceptable derivatives include in particular prodrugs of the compounds of formula (I) which are reconverted in vivo to the compounds of formula (I) or an active form thereof (metabolite). Examples are hydrolysable esters of the compounds of formula (I) wherein the third of radicals R1, R2, R3 represents an acidic residue, e.g. alkyl (the third of radicals R1, R2, R3 comprising the functionality COOAlkyl), aralkyl (the third of radicals R1, R2, R3 comprising the functionality COOAlkaryl, e.g., COOAlkPhenyl), pivaloyloxymethyl, acetoxymethyl, phthalidyl, indanyl and methoxymethyl esters thereof.

The term "proton pump inhibitor" as used herein will be understood to refer to those compounds which inhibit the gastric H^+,K^+-ATPase activity. This activity can be determined in well-known assays, e.g. in terms of inorganic phosphate release from the substrate ATP (Hongo T. et al., J.J. Pharmacol. 52: 295 (1990)). Preferred are those compounds of Formula (I) having a half-maximal inhibitotry concentration (IC_{50}) of 50 µM or less. Using compounds of Formula (I) having an IC_{50} of 5 µM or less may be of advantage according to the invention. Very effective proton pump inhibitors of Formula (I) have an IC_{50} of 0,5 µM or less. According to a further aspect, those compounds of Formula (I) are preferred which reversibly inhibit the proton pump. According to still a further aspect, those compounds of Formula (I) are preferred whose proton pump-inhibiting actiivity is essentially pH-independent over a pH range of about 3 to 8.

Such compounds can be identified among the compounds of Formula (I) using well-known screening procedures such as high-throughput screening (HTS) procedures. A typical procedure comprises testing the gastric H^+,K^+-ATPase inhibition by each of a number of candidate compounds of Formula (I) and identifying those which have the desired activity. It will also be appreciated that such proton pump inhibiting compounds may also possess anti-inflammatory action.

Thus, according to a particular aspect, the present invention relates to the use of proton pump inhibitors which are selected among the compounds of Formula (I). These compounds of Formula (I) are effective as gastric acid secretion inhibitors.

In a more general sense, the compounds can be used for prevention and treatment of gastric-acid related conditions in mammals and especially in man, such

as gastritis, duodenitis, ulceration, especially gastric ulcer and duodenal ulcer, reflux esophagitis and Zollinger-Ellison syndrome. Gastritis may be erosive or non-erosive, acute or chronic. In particular, the compounds are useful as antiulcer agent.

Furthermore, the compounds can be used for the treatment of other, especially gastrointestinal, disorders where the gastric acid-inhibitory effect is desirable e.g. in patients on NSAID therapy, in patients with Non Ulcer Dyspepsia, in patients with symptomatic gastro-esophageal reflux disease, and in patients with gastrinomas. The compounds of the invention may also be used in patients in intensive care situations, in patients with acute upper gastrointestinal bleeding, pre- and postoperatively to prevent aspiration of gastric acid and to prevent and treat stress ulceration. Further, the compound of the invention may be useful in the treatment of psoriasis as well as in the treatment of Helicobacter infections and diseases related to these. The compound of the invention may also be used for treatment of inflammatory conditions in mammals, including man.

According to a particular aspect, the invention relates to the use of gastroprotective compounds of Formula (I). The term "gastroprotective" as used herein will be understood to refer to those compounds which are in particular capable of reducing the sensitivity of the gastrointestinal mucosa and in particular the gastric mucosa to topical injury. Such topical injury may be caused by irritants. Accordng to a particular aspect of the invention said irritants are selected from the group consisiting essentially of NSAIDs and COX-inhibitors.

The earliest gross pathologic finding in ulcerogenesis is the erosion of the gastric epithelial monolayer. With progression of said erosion the integrity of the gastric mucosa thins, and segments of the gastrointestinal mucosa penetrate through the muscularis mucosa.

Changes in the normal mucosa defense and repair by certain factors such as NSAIDs and H. pylori, also play a role in the pathology of peptic ulcer disease.

According to one aspect of the present invention, the use of compounds of Formula (I) is directed to treating or preventing acid-mediated mucosal damage and subsequent ulceration. In particular, said use is directed to treating or preventing the pathologic changes involved therewith.

The present invention provides methods of treatment, and pharmaceutical compositions useful therein as well as suitable packaging therefor, which are especially applicable to mammals which suffer from or in the future may suffer from injury, damage or lesions of gastrointestinal mucosa.

Using the compounds of Formula (I) has particular advantages over other NSAIDs, especially those more established in use, which may actually exacerbate the progress of mucosal lesions und in particular gastric ulceration, especially when long-term application is indicated. It is surprising that the compounds of Formula (I) are useful in treating or preventing such mucosal lesions und in particular gastric ulceration.

The ability of the compounds of formula (I) to prevent and to reverse the disease process which ultimately leads to mucosal destruction has far-reaching implications for the safe and effective treatment of mammals, especially those which need long-term anti-inflammatory therapy.

As used herein, the term "mammal(s)" denotes any mammal, preferably humans, cat, dog or horse, of which there are a large number of different breeds.

In accordance with the present invention, treating or preventing gastric acid-related conditions of a mammal in need of such treatment, comprises administering to said mammal an amount therapeutically effective for treating or preventing said conditions, of one or more than one compound of Formula (I).

Said treatment or prevention especially comprises ameliorating, diminishing, actively treating, reversing or preventing any gastric acid-related condition; e.g. injury, damage or lesions, of gastrointestinal and especially gastric mucosa. The expression "treating or preventing" as used herein with reference to the administration of the gastroprotective compounds of the present invention, is intended to refer to both the therapeutic objective of said administration as well as the therapeutic results actually achieved by said administration. As above-discussed, the extent of therapy accomplished by administration of said compounds may range from an amelioration to a significant diminishing of the course of the disease, and beyond to active treatment of the disease, including a reversal of the disease process.

Treating or preventing gastric acid-related conditions may also comprise administering in addition to one or more than one compound of Formula (I), one or more members selected from the group consisting essentially of antibacterially active agents, further gastroprotective agents such as further proton inhibitors and H_2-receptor antagonists, antacid agents, alginates and prokinetic agents.

Combinations with antibacterially active agents are especially useful in treating H. pylori-positive individuals (anti-H. pylori therapy). Antibacterially active agents such as clarithromycin plus metronidazole or amoxicillin in combination with proton pump inhibitors yield high eradication rates in respect of H. pylori infections. Further suitable antibacterially active agents which may be mentioned are ß-lactam antibiotics, for

example penicillins (such as benzylpenicillin, phenoxymethylpenicillin, propicillin, azidicillin, dicioxacillin, flucloxacillin, oxacillin, amoxicillin, bacampicillin, ampicillin, meziocillin, piperacillin or aziocillin), cephalosporins (such as cefadroxil, cefaclor, cefalexin, cefalexim, cefuroxim, cefetamet, cefadroxil, ceftibuten, cefpodoxim, cefotetan, cefazolin, cefoperazon, ceftizoxim, ceftaxim, ceftazidim, cefamandol, cefepim, cefoxitin, cefodizim, cefsulodin, ceftriaxon, cefotiam or cefmenoxim) or other ß-lactam antibiotics (e.g. aztreonam, loracarbef or meropenem); enzyme inhibitors for example sulbactam, tetracyclines, for example tetracycline, oxytetracycline, minocycline or doxycycline aminoglycosides, for example tobramycin, gentamicin, neomycin, streptomycin, amikacin, netilmicin, paromomycin or spectinomycin; amphenicols, for example chloramphenicol or thiamphenicol; lincomycins and macrolide antibiotics, for example clindamycin, lincomycin, erythromycin, clarithromycin, spiramycin, roxithromycin or azithromycin; polypeptide antibiotics, for example colistin, polymixin B, teioplanin or vancomycin, gyrase inhibitors, for example norfloxacin, cinoxacin, ciprofloxacin, pipemidic acid, enoxacin, nalidixie acid, pefloxacin, fieroxacin or ofloxacin; nitroimidazoles, for example metronidazole; or other antibiotics, for example fosfomycin or fucidic acid, where these antibacterially active substances are administered on their own or alternatively can be combined with one another.

The following compounds may be primarily mentioned as further proton pump inhibitors: omeprazole, lansoprazole, rabeprazole, leminoprazole, nepaprazole and pantoprazole.

The compounds of Formula (I) of the present invention may also be combined with other therapeutically active ingredients which would be readily apparent to the skilled artisan in this field, and which will usually be determined by the circumstances under which the therapeutic agent of the present invention is administered. Examples of such other therapeutically active ingredients include, but are not limited to the above agents.

Further examples of such other therapeutically active ingredients include anti-inflammatory agents, in particular NSAIDs and additional classes of anti-inflammatory agents and examples thereof include, *e.g.*, H_1-receptor antagonists; kinin-B_1- and B_2-receptor antagonists; prostaglandin inhibitors such as PGD-, PGF- PGI_2 -, and PGE-receptor antagonists; thromboxane A_2 (TXA2-) inhibitors; PAF-receptor antagonists; gold in the form of an aurothio group together with various hydrophilic groups; immunosuppressive agents, *e.g.*, cyclosporine, azathioprine, and methotrexate; anti-inflammatory glucocorticoids, *e.g.*, dexamethasone; broad-spectrum antiparasitic

antibiotics, *e.g.*, the avermectins and the milbemycins; penicillamine; hydroxychloroquine; anti-gout agents, *e.g.*, colchicine, xanthine oxidase inhibitors, *e.g.*, allopurinol, and uricosuric agents, *e.g.*, probenecid, sulfinpyrazone, and benzbromarone.

According to a particular aspect of the instant invention, one or more than one compound of Formula (I) is combined with an ulcerogenic anti-inflammatory agent. Anti-inflammatory agents are ulcerogenic if they inhibit the cyclooxygenase, in particular the cyclooxygenase 1, and as a consequence, the production of certain prostaglandins, in particular prostaglandin E_2. Ulcerogenic anti-inflammatory agents that belong to the class of NSAIDs are preferably combined with one or more than one compound of Formula (I). Particular ulcerogenic anti-inflammatory agents which may be mentioned are acetylsalicylic acid (ASA), sodium salicylate, acetaminophen, phenacetin, ibuprofen, ketoprofen, indomethacin, flurbiprofen, diclofenac, naproxen, piroxicam, tebufelone, etodolac, nabumetone, tenidap, alcofenac, antipyrine, amimopyrine, dipyrone, animopyrone, phenylbutazone, clofezone, oxyphenbutazone, prexazone, apazone, benzydamine, bucolome, cinchopen, clonixin, ditrazol, epirizole, fenoprofen, floctafenine, flufenamic acid, glaphenine, indoprofen, meclofenamic acid, mefenamic acid, niflumic acid, salidifamides, sulindac, suprofen, tolmetin, nabumetone, tiaramide, proquazone, bufexamac, flumizole, tinoridine, timegadine, dapsone, diflunisal, benorylate, fosfosal, fenclofenac, etodolac, fentiazac, tilomisole, carprofen, fenbufen, oxaprozin, tiaprofenic acid, pirprofen, feprazone, piroxicam, sudoxicam, isoxicam, celecoxib, tenoxicam. According to a particular embodiment, acetylsalicylic acid is combined with one or more than one compound of Formula (I).

Accordingly, this type of medication provides a means for effectively treating inflammatory conditions while having gastric sparing properties. This is particularly advantegous in situations where the administration of the ulcerogenic anti-inflammatory agent involves the gastrointestinal tract, e.g. in case of oral administration or gastrointestinal inflammatory conditions.

In accordance with a regimen which would be used according to the invention, it is contemplated that the compounds of Formula (I) would be administered in combination with other medications used on a regularly scheduled basis. It is also envisioned that administration in combinations could assume a number of different forms and still be within the scope of the present invention. For example, the compounds of Formula (I) might simply be formulated with one or more of the other therapeutic agents which are to form the intended combination, into a convenient dosage form, such as an oral tablet, containing all of the drugs forming the

combination. Varying half-lives for the different drugs could be accommodated by the person skilled in preparing formulations by creating controlled-release forms of said drugs with different release times so that relatively uniform dosing was achieved. A medicated feed used as the dosage form could also be prepared in accordance with well known principles in the art of formulation, in which the drugs used in the combination were simply present together in admixture in the feed composition. The present invention also contemplates co-administration in which the combination of drugs is achieved by the simultaneous administration of the drugs to be given in combination. Such co-administration could even be by means of different dosage forms and routes of administration. The present invention further contemplates the use of such combinations in accordance with different but regular and continuous dosing schedules whereby desired plasma levels of the drugs involved were maintained in the mammal being treated, even though the individual drugs making up the combination were not being administered to said mammal simultaneously. All such combinations would be well within the skill of the art to devise and administer.

When the compounds of Formula (I) are to be used as active ingredients in the methods and compositions of the present invention, they can be incorporated into standard pharmaceutical dosage forms. Thus, the present invention also relates to pharmaceutical compositions comprising a pharmaceutically acceptable carrier and an amount therapeutically effective for inhibiting the proton pump, of a compound of Formula (I) as above-defined. For example, they are useful when administered in systemic or local, oral or parenteral applications and for this purpose are combined with the usual pharmaceutical excipients, diluents and adjuvants, *e.g.*, organic and inorganic inert carrier materials such as water, gelatin, lactose, starch, magnesium stearate, talc, vegetable oils, gums, polyalkyleneglycols, *etc.* These pharmaceutical preparations can be employed in a solid form, *e.g.*, as tablets, capsules, and especially in combination with or for admixture with a palatable food item suitable for mammals; or they can be administered in liquid form, *e.g.*, as solutions and elixirs. Pharmaceutical excipients and adjuvants which can be added include preservatives, antioxidants, antimicrobial agents and other stabilizers; wetting, emulsifying, and suspending agents, and anticaking compounds; fragrance and coloring additives; compositions for improving compressibility, or to create a delayed-, sustained-, or controlled-release of the active ingredient; and various salts to change the osmotic pressure of the pharmaceutical preparation or to act as buffers. Particular dosage forms which have been used with success include a 5% mixed-micelle solution of ML3000 for intravenous injection, a 3% palatable paste, and oral tablets.

The therapeutically effective amount of a compound of Formula (I) as defined may be administered systemically to said mammal, wherein said systemic administration comprises: (1) injection or infusion into suitable body tissues or cavities of a pharmaceutical composition containing said compound in suitable liquid form such as aqueous solutions, emulsions or suspensions for intraarterial, intra- or transdermal (including subcutaneous), or intraspinal especially intrathecal and most commonly intramuscular or intravenous delivery thereof; or for serving as a depot for delivery thereof; (2) instillation into suitable body tissues or cavities of a pharmaceutical composition containing said compound in suitable solid form, e.g., comprising a matrix of bio-compatible and bio-erodible materials in which particles of a solid gastroprotective compound of Formula (I) are dispersed, or in which, possibly, globules or isolated cells of a liquid gastroprotective compound of Formula (I) are entrapped, for serving as a solid implant composition for delayed-, sustained-, and/or controlled-release delivery thereof; or (3) ingestion or administration of a pharmaceutical composition containing said compound in suitable solid or liquid form for transdermal delivery thereof, for instance a transdermal patch or a subepidermal (subcuticular) implant, for peroral delivery thereof.

A substantial number of the dosage forms described herein may be formulated so as to provide controlled-, sustained-, and/or delayed release of the active ingredient from said dosage form.

A useful controlled release dosage form of ML3000 in accordance with the present invention is one which maintains a ML3000 plasma level greater than 100 ng/mL for most of the day after a single oral dose at 5 mg/kg. Preferred oral controlled release dosage forms of ML3000 in accordance with the present invention are ones which maintain a plasma ML3000 concentration greater than 100 ng/mL for a period of time greater than that for which an immediate release dosage form of ML3000 maintains a comparable plasma level, when said immediate release dosage form and controlled release dosage form are administered at the same dose.

Immediate release ML3000 dosage forms containing doses of 2.5 and 5 mg/kg maintain a plasma ML3000 concentration above 100 and 200 ng/mL for 8 hours, respectively.

Preferred peroral dosage forms for systemic administration are solids, *e.g.*, palatable oral compositions such as fast dissolving palatable wafers, tablets, capsules, caplets, *etc.*, and liquids, *e.g.*, solutions, suspensions, emulsions, *etc.* Pharmaceutical compositions of special types suitable for oral administration to mammals may be used, and include, but are not limited to such items as an oral paste to be delivered to

the back of the tongue of the mammal being treated, a granular form to be delivered through incorporation in the mammal's food, and a chewable form wherein the active ingredient is consumed along with the palatable chew, or a chewable form which may deliver the active ingredient by leaching from the body of the chew which is not consumed, during mastication by the mammal being treated.

Said therapeutically effective amount of a compound of Formula (I) as defined may also be administered locally to said mammal, wherein said local administration comprises: (1) injection or infusion into a local site of gastric acid-related condition of a pharmaceutical composition containing said compound of formula (I) in suitable liquid form for delivery thereof, including components which provide delayed-release, controlled-release, and/or sustained-release of said compound into said local site; or for serving as a depot for delivery thereof wherein said composition provides storage of said compound and thereafter delayed-, sustained-, and/or controlled-release thereof; or (2) instillation of a pharmaceutical composition containing said compound in suitable solid form for serving as a solid implant for delivery thereof, said composition optionally providing delayed-, sustained-, and/or controlled-release of said compound to said local site.

Injections may also be made of pharmaceutical compositions containing the gastroprotective compound of Formula (I), where the pharmaceutical composition is in delayed-release, controlled-release, or sustained-release form. These formulations of recognized composition may be a solids, semi-solids, gels or other liquid/solid combinations in which an erodible matrix or series of coatings is used to provide a continuous release of the compound of Formula (I) at a predetermined rate or at variable rates if desired. The terms "extended-release" and "long-acting" as well as others are used to describe these formulations. All of these employ various combinations of bioerodible polymers, *e.g.*, various cellulosic polymers, and natural materials, *e.g.*, corn starch and magnesium stearate, to obtain slow and/or uniform dispensing of the compound of Formula (I) contained within the matrix.

The therapeutically effective amount for treating or preventing gastric acid-related diseases, of the compound of Formula (I), is administered to a mammal being treated in an amount expressed as milligrams per kilogram of body weight of said mammal, per day: "mg/kg/day". The expression "per day" as used herein should not be interpreted as necessarily requiring that any particular dosage form be administered on a daily basis to the mammal being treated. The expression "per day" is merely an indication of the smallest convenient but arbitrary segment of time which is being used as part of the overall unit for measuring the dose of gastroprotective

compound being administered. The dose, *i.e.*, the therapeutically effective amount of a compound of Formula (I) for treating or preventing gastric acid-related diseases will usually range from about 0.1 mg/kg/day to about 20.0 mg/kg/day, preferably from about 0.1 mg/kg/day to about 12.0 mg/kg/day, more preferably from about 0.5 mg/kg/day to about 10.0 mg/kg/day, and most preferably from about 0.5 mg/kg/day to about 8.0 mg/kg/day. Typical dosage forms and amounts for ML3000 would include oral administration of ML3000 at a dose rate of 2.5-5.0 mg/kg/day of body weight. Special requirements may be needed for patients having Zollinger-Ellison syndrome, such as a need for higher doses than the average patient.

It is necessary for the skilled artisan, not only to determine the preferred route of administration and the corresponding dosage form and amount, but said artisan must also determine the dosing regimen, *i.e.*, the frequency of dosing. In general terms it is most likely that the choice will be between once-a-day (*s.i.d.*) dosing and twice-a-day (*b.i.d.*) dosing, and that the former will provide more rapid and profound therapy, while the latter will provide less profound but more sustained therapy. However, this generalization does not take into account such important variables as the specific type of gastric acid-related disease involved, the specific therapeutic agent involved and its pharmacokinetics, and the specific patient (mammal) involved. For an approved product in the marketplace, much of this information is already provided by the results of clinical studies carried out to obtain such approval. In other cases, such information may be obtained in a straightforward manner in accordance with the teachings and guidelines contained in the instant specification taken in light of the knowledge and skill of the artisan. The results which are obtained can also be correlated with data from corresponding evaluations of an approved product in the same assays.

It is also contemplated that in accordance with the present invention there will also be provided a package suitable for use in commerce for treating or preventing gastric acid-related diseases in a mammal in need of such treatment, comprising a suitable outer carton and an inner container removably housed therein; enclosed in said container a suitable dosage form of a compound of Formula (I) as described hereinabove; and associated with said carton or container printed instructional and informational material, which may be attached to said carton or to said container enclosed in said carton, or displayed as an integral part of said carton or container, said instructional and informational material stating in words which convey to a reader thereof that said active ingredient, when administered to a mammal in a condition of gastric acid-related disease, will ameliorate, diminish, actively treat, reverse or prevent

any injury, damage or lesions of gastrointestinal mucosa. In a preferred embodiment said package comprising carton and container as above-described will conform to all regulatory requirements relating to the sale and use of drugs for the treatment of animals, including especially said instructional and informational material.

It is also contemplated that in accordance with the present invention there will further be provided a package of the type described immediately above, comprising a suitable container as described; enclosed in said container an oral dosage form of a compound of Formula (I); and associated with said container printed instructional and informational material as above-described.

The method of the present invention can be further defined to comprises two basic steps: (I) establishing the status of a candidate mammal as presently or prospectively being in a condition of gastric acid-related disease, thereby confirming that said mammal is in need of such treatment; and thereupon (II) treating or preventing said condition by administering to said mammal an amount therapeutically effective for treating or preventing said gastric acid-related disease, of a gastroprotective compound of Formula (I). The various aspects of Step (II) have already been discussed above in detail. Accordingly, the aspects of Step (I) will now be discussed in detail.

As far as diagnosis is concerned, it is expedient to establish the status of a mammal which is a candidate for treatment in accordance with the present invention as to whether or not the mammal is presently or prospectively in a condition of gastric acid-related disease. The expression "presently or prospectively" as used herein is intended to mean that in accordance with the below-discussed methods of making that determination, it is possible to identify a candidate mammal as either being presently in need of such treatment, or as very likely or expected to be in need of such treatment in the short term future. Prospective need of treatment may be established by those determinations of positive factors which from the experience of the artisan lead directly to the condition of gastric acid-related disease. For example, the artisan may establish from clinical examination of a mammal that it has a gastric acid-related disease, and may confirm this conclusion with further evidence from which it may be determined in accordance with established methods of measurement that the mammal will develop a gastric acid-related disease within the short term future.

The status of said mammal as presently or prospectively being in said condition of gastric acid-related disease, and thus in need of such treatment, is in particular determined by positive results from the clinical examination and evaluation of the gastrointestinal tract of the candidate mammal, e.g. by noninvasive procedures

including fiberoptic endoscopy, magnetic resonance imaging (MRI) and radiographic methods such as double-contrast barium x-ray. Other clinical symptomology and signs would include those gained from direct examination of the gastric mucosa of the candidate mammal, for example by means of biopsy.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Effect of ML 3000 on pig microsomal H,K-ATPase activity;

Figure 2: Effect of omeprazole on pig microsomal H,K-ATPase activity;

Figure 3: Effect of pH on ML 3000 inhibition of gastric H,K-ATPase;

Figure 4: Effect of ML 3000 dilution on inhibition of gastric H,K-ATPase;

Figure 5: Effect of arachidonic acid on pig microsomal H,K-ATPase activity.

Figure 6: Effect of PGE_2 on pig microsomal H,K-ATPase activity;

Figure 7: Effect of TEDBC on pig microsomal H,K-ATPase activity;

Figure 8: Effect of ZD-2138 on pig microsomal H,K-ATPase activity;

Figure 9: Effect of acetyl salicylic acid on pig microsomal H,K-ATPase activity.

Figure 10: Effect of NS 398 on pig microsomal H,K-ATPase activity;

Figure 11: Effect of naproxen on pig microsomal H,K-ATPase activity;

Figure 12: Effect of indomethacin on pig microsomal H,K-ATPase activity;

Figure 13: Effect of leukotriene B4 on pig microsomal H,K-ATPase activity;

Figure 14: Effect of leukotriene D4 on gastric H,K-ATPase;

Figure 15: Effect of ML 3000 on histamine-stimulated acidification in rabbit gastric glands;

Figure 16: Effect of ML 3000 on forskolin-stimulated acidification in rabbit gastric glands;

Figure 17: Effect of ML 3000 on baseline IL-8 secretion by human gastric epithelial cells;

Figure 18: Effect of ML 3000 on IL-8 secretion by IL-1ß-stimulated human gastric epithelial cells;

Figure 19: Effect of ZD 2138 on baseline IL-8 secretion by human gastric epithelial cells;

Figure 20: Effect of ZD 2138 on IL-8 secretion by IL-1ß-stimulated human gastric epithelial cells.

DESCRIPTION OF PREFERRED EMBODIMENTS

In order to further demonstrate the methods and compositions of the present invention, there is presented in the paragraphs which follow specific descriptive examples of typical procedures which may be employed in carrying out said methods. However, said examples are intended to be illustrative only and should not be taken as in any way a limitation of the present invention, for which purpose the present claims are appended hereto.

EXAMPLE 1

Effects of ML 3000 on gastric microsomal K^+-stimulated, SCH28080-sensitive H,K-ATPase activity.

Strategy. Microsomal gastric H,K-ATPase was prepared from pig gastric mucosal homogenates by differential centrifugation. Briefly, pig stomachs obtained at a slaughter-house within 1 hour post mortem were washed with ice-cold 0,25 M sucrose and the fundus was dissected from the cardiac and antral regions. All subsequent prodedures were at 4 °C. The mucosa was flooded with saturated NaCl, and the surface mucus and superficial cells wiped off with paper towels. The mucosa was scraped from the underlying connective tissue, suspended (10 % w/v) in isolation buffer (0,25 M sucrose, 20 mM HEPES, pH 7,4, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride), and disrupted by two 10 second bursts at maximum power in a Tissumizer (Tekmar, Cincinnati, OH). The homogenate was centrifuged at 20,000 g for 30 minutes, and the supernatant was centrifuged at 10^5 g for 1 hour. The resulting microsomal pellet was resuspended in isolation buffer, and applied to a discontinuous gradient of 7 % w/v Ficoll and 34 % sucrose (both in isolation buffer). After 3 hours at 32,500 rpm (Sorvall AH 629 rotor), the microsomal band (G1) recovered from the 7 % Ficoll interface was resuspended to 10 mg/ml in 15 mM PIPES-Tris, pH 6,8, diluted 1:1 with cold 60 % sucrose, lyophilized in 0,5 ml aliquots, and stored at -70 °C. GI microsomes were about 0,1 microns in diameter, were enriched in H.K.-ATPase activity, and more than 80 % of their protein content banded at 94 kDa by SDS-PAGE. ATP hydrolytic activity of microsomes was quantitated in terms of inorganic phosphate release from substrate ATP and measured in graded concentrations (ranging from 10^{-9}M to 10^{-4}M) of ML 3000, and a wide range of other compounds. Reaction mixtures for assay of K^+-stimulated ATPase activity in pig

gastric microsomal membranes contained 5 µg membrane protein, 100 mM Tris-acetate, pH 7.0, 1mM $MgCl_2$, 1 mM NaNa, 0,1 mM EGTA, 5 µM ATP (γ-^{32}P-ATP, 10 Ci/mmol, NEN, Boston, MA), 0–10 mM KCl, and 0–100 µM SCH 28080. After 20 minutes incubation at 37 °C, reactions were stopped by addition of 10 % w/v activated charcoal (Sigma), 5,5 % w/v trichloroacetic acid, vortexed vigorously, and centrifuged at 14,000 g for 10 minutes at 4 °C. Inorganic phosphate (γ-^{32}Pi) content of the supernatant was measured by scintillation counting. Specific H,K-ATPase activity was calculated as the difference in microsomal ATPase activities in the presence and absence of the specific gastric H,K-ATPase inhibitor SCH28080, and was expressed as mmoles Pi/mg protein/hr; graphical depiction of the data shows percent inhibition of H,K-ATPase activity as a function of compound concentrations. Graphs with standard error bars present inter-assay data variance in three independent assays in each of which ATPase activities in three separate but identical reaction conditions were measured. Graphs without standard error bars represent the mean ATPase activities in three separate but identical reaction conditions in one of at least three independent assays; typical data are shown in these cases.

Results. Gastric H,K-ATPase activity was dose-dependently inhibited by ML 3000, with a half-maximal inhibitory concentration (IC_{50}) of 15 µM (Figure 1). The inhibitory activity of ML 3000 was compared to that of a classical proton pump inhibitor (PPI), the substituted benzimidazole omeprazole. Figure 2 shows the effect of omeprazole on H,K-ATPase activity under assay conditions identical to those in Figure 1; the estimated IC_{50} for omeprazole was 1 µM. These data show that ML 3000 and omeprazole display comparable IC_{50} with respect to gastric H,K-ATPase activity, at least in the setting of this particular in vitro assay under the specified conditions. Published IC_{50} for omeprazole with respect to gastric proton pump activity range from 470 nM to 36 µM depending on the conditions of the assay (3,4,5). For other PPIs, picoprazole IC_{50} is 2 µM (6), rabeprazole IC_{50} is 72 nM (3), and lansoprazole IC_{50} is 2.1 µM (7).

The wide range of published PPI IC_{50} values for microsomal H,K-ATPase reflects the mechanistic necessity for compound acidification to allow formation of a thiol-reactive sulfoxide intermediate which then irreversibly derivatizes H,K-ATPase a subunit cysteine residues leading to enzyme inhibition. Omeprazole at neutral or higher pH exerts no inhibitory effects on gastric H,K-ATPase. Microsomal vesicle preparations vary widely in their ion-tightness, which affects the extent to which internal pH can be lowered by H,K-ATPase turnover, and therefore the extent to which

omeprazole diffusing into the vesicle can be acidified and activated. Alternatively, prior in vitro acidification of omeprazole ensures induction of its inhibitory properties, and is essential in assays carried out using ion-permeable microsomal H,K-ATPase preparations. For this reason, the omeprazole inhibitory data shown in Figure 2 were derived using omeprazole acidified to pH 6.1 and incubated with the enzyme for 30 min at the same pH (8).

To determine whether ML 3000 displayed comparable acid-activation properties, a half-maximal inhibitory concentration of ML 3000 was titrated to different pHs and the effects on H,K-ATPase activity were measured. As shown in Figure 3, acidification of ML 3000 had no significant effect on its H,K-ATPase inhibitory profile. These data indicate that although ML 3000 and omeprazole have comparable IC_{50} for H,K-ATPase, ML 3000 unlike omeprazole does not require acidification for induction of inhibitory activity.

Given that PPIs are irreversible inhibitors of H,K-ATPase activity, covalently binding to the catalytic α-subunit, it was determined whether ML 3000 inhibition of H,K-ATPase was reversible or irreversible. Gastric H,K-ATPase-enriched microsomes were treated with a maximally-inhibitory concentration of ML 3000 and then diluted with a large excess of buffer to reduce the ML 3000 concentration from 100 µM to 3.3 µM. The results, shown in Figure 4, indicated that dilution of ML 3000 restored H,K-ATPase activity, and are consistent with ML 3000 inhibiting H,K-ATPase activity in a reversible manner, ie., ML 3000 does not covalently derivatize either sub-unit of the gastric H,K-ATPase in vitro.

Arachidonic acid and prostaglandin E2 (PGE_2) also dose-dependently inhibited H,K-ATPase activity, with IC_{50} of 30 µM and 45 µM respectively (Figures 5 and 6). The PGE2 data contradict a previous study in which no inhibitory effect of PGE_2 on pig gastric H,K-ATPase (9) was found. Differences in the specific ATPase assay used in that study may account for this discrepancy. Since ML3000 and arachidonic acid are anionic amphiphiles, their inhibitory effects could result from specific interactions with H,K-ATPase sub-unit binding sites, or from less-specific hydrophobic interactions with H,K-ATPase-associated microsomal membrane lipids, or a combination of both factors.

Since ML 3000 also shows 5-lipoxygenase inhibition, the effects of two lipoxygenase inhibitors on microsomal H,K-ATPase activity were studied. Figure 7 shows that 2-(1-thienyl)ethyl 3,4-dihydroxybenzylidenecyanoacetate (TEDBC), a powerful inhibitor of 5-, 12-, and 15-lipoxygenses, inhibited microsomal H,K-ATPase activity with an IC_{50} of 3.3 µM. In contrast, the 5-lipoxygenase-specific inhibitor 6-((3-

fluor-5-(methoxy-3,4,5,6-tetrahydro-2H-pyran-4-yl)phenoxy)methyl)chinolin (ZD-2138) had a minimal effect on H,K-ATPase activity (~20% inhibition at 10^{-5} M) (Figure 8). These data are consistent with gastric microsomal 12- and 15-lipoxygenases playing a role in H,K-ATPase activation, or with direct interaction of TEDBC with H,K-ATPase subunits altering enzyme conformation and hence activity.

In order to compare the H,K-ATPase inhibitory effect of ML 3000 with other NSAIDs, the effects of acetyl salicylic acid, naproxen, indomethacin and a selective COX-2 inhibitor, NS 398, on proton pump activity were measured. All four NSAIDs were without inhibitory effects on microsomal H,K-ATPase activity at concentrations up to 10^{-4} M (10^{-3} M in the case of acetyl salicylic acid) (Figures 9, 10, 11 and 12). Indomethacin was previously reported to inhibit gastric H,K-ATPase at somewhat higher concentration (Ki = 0.67 x 10^{-3} M) (10). This data clearly differentiate ML 3000 from other NSAIDs in terms of inhibitory effect on gastric H,K-ATPase activity.

In order to establish whether the ML 3000 inhibition of gastric H,K-ATPase reflected the compound's effects on putative functional leukotriene metabolic pathways present in pig gastric microsomes, the effects of leukotriene B4 and D4 on H,K-ATPase activity were studied. Solubility issues precluded studying LTB4 or LTD4 concentrations greater than 1 µM. As shown in Figures 13 and 14, neither leukotriene showed any inhibitory activity against H,K-ATPase at physiological concentrations (10^{-9}-10^{-8} M); only at non-physiological concentrations greater than 10^{-7} M was there any significant attentuation of H,K-ATPase activity.

EXAMPLE 2

Effects of ML 3000 and other compounds on gastric parietal cell histamine-stimulated acid accumulation.

Strategy. Gastric parietal cells were isolated from New Zealand White Rabbits by pronase/collagenase digestion of fundic mucosa followed by enrichment of cells on discontinuous Nycodenz gradients in a manner known per se. Aminopyrine accumulation into parietal cells was assessed in 96 well filter plates with Durapore membranes. Briefly, cells were preincubated with [^{14}C]-aminopyrine and then 100,000 cells/200 µl per well were incubated without or with test compounds for 15 minutes prior to incubation for a further 30 min in the absence or presence of 100 µM histamine. All determinations were performed in quadruplicate. Basal aminopyrine accumulation was determined as aminopyrine accumulation into untreated cells subtracted from accumulation in the presence of KSCN (a reflection of non-specific isotope trapping). Graphical depiction of the data shows percent inhibition of

histamine-stimulated acid accumulation by the cells as a function of compound concentrations.

Results. ML 3000 dose-dependently inhibited histamine-stimulated acid accumulation by rabbit gastric parietal cells, with a half-maximal inhibitory concentration (IC_{50}) of 40 µM (Figure 15). ML 3000 also dose-dependently inhibited forskolin-stimulated acid accumulation by rabbit gastric parietal cells, with a half-maximal inhibitory concentration (IC_{50}) of ~45 µM (Figure 16). These data indicate that ML 3000 affects parietal cell acid-secretory mechanisms downstream of cAMP mobilization induced by histamine H2 receptor activation. The data are consistent with ML 3000 inhibition of parietal cell acid secretion resulting from direct interaction of ML 3000 with the gastric H,K-ATPase. However, the discepancy between ML 3000 IC_{50} in microsomal vesicles (15 µM) and in isolated parietal cells (40-45 µM) suggests that ML 3000 access to the intracellular H,K-ATPase compartment in parietal cells may be slowed by permeability constraints at the plasma membrane.

Also, as was found with microsomal H,K-ATPase, other NSAIDs such as acetyl salicylic acid, naproxen, and NS 398 (up to concentrations of 10^{-4} M) had no effect on acid accumulation by isolated rabbit parietal cells.

EXAMPLE 3

Effects of ML 3000 on IL-1ß-induced and Heliobacter pylori-induced IL-8 secretion in human gastric adenocarcinoma (AGS) cells.

Strategy. AGS cells were incubated with test compounds, challanged with IL-1ß, and sebsequent secretion of IL-8 into the culture medium was measured by enzyme linked immunosorbent assay; graphical depiction of the data shows precent inhibition od unstimulated or stimulated IL-8 secrretion as a function of a compound concentrations.

Results. Without stimulation by IL-1ß, and in the absence of ML3000 or ZD2138, AGS cells (5 x 104 in µl culture medium) secreted IL-8 over a period of 6 hr to a concentration of ~225 pg/ml (Figures 17 and 19). When stimulated by IL-1ß (20 ng/ml), AGS cell IL-8 secretion over a period of 6 hr was increased ~27-fold, to a concentration of ~6000 pg/ml (Figures 18 and 20). ML3000 inhibited both baseline (Figure 17) and IL-1ß-stimulated IL-8 secretion (Figure 18), with IC_{50} of 0.75 µM and 30 µM repectively.

The 5-lipoxygenase-specific inhibitor (ZD-2138), which was withour effect on microsomal H^+,K^+-ATPase activity, showed a dose-dependent inhibition of baseline IL-8 secretion by AGS cells (Figures 19), with an IC50 of ~0.4 µM. In contrast, ZD-2138

was without effect on IL-1ß-stimulated IL-8 secretion by AGS cells (Figure 20). H^+,K^+-ATPase inhibition by ML3000, which was demonstrated above, does not underlie IL-8 secretory inhibtion in this model since AGS cells do not express H^+,K^+-ATPase.

To the extent that IL-8 is a potent inflammatory mediator in the gastric mucosa, the finding that ML3000 profoundly inhibits baseline and IL-1ß-stimulated IL-8 secretion in gastric epithelial cells suggests the inhibtion is not effected by the 5-lipoxygenase inhibitory activity of ML3000.

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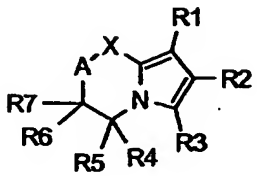
WE CLAIM

1. The use of one or more than one compound of Formula (I):



wherein

X represents
CR8R9, S, O, NR12 or C(O);

A represents
CR10R11 or a bond between X and the atom carrying radicals R6 and R7;

the first of radicals R1, R2, R3 represents
aryl, optionally substituted with one or more than one substituents independently selected among the group consisting of halogen, alkyl, halogenoalkyl, alkoxy, aryloxy, halogenoalkoxy, alkylthio, hydroxy, nitro, alkylsulfinyl, alkylsulfonyl, sulfamoyl, N-alkylsulfamoyl, N,N-di-alkylsulfamoyl, alkylsulfonamido and alkylsulfon-N-alkylamido; or
an aromatic or non-aromatic, mono- or bicyclic, optionally benzoannellated, heterocyclic group having 1, 2 or 3 heteroatoms independently selected from N, O and S and optionally being substituted with one or more than one substituents independently selected among the group consisting of halogen, alkyl, halogenoalkyl, alkoxy, aryloxy, halogenoalkoxy, alkylthio, hydroxy, nitro, alkylsulfinyl, alkylsulfonyl, sulfamoyl, N-alkylsulfamoyl, N,N-di-alkylsulfamoyl, alkylsulfonamido and alkylsulfon-N-alkylamido;

the second of radicals R1, R2, R3 represents
alkyl, optionally substituted with one or more than one substituents independently selected among the group consisting of halogen, cycloalkyl, alkoxy, trifluormethoxy, hydroxy and trifluormethyl;

cycloalkyl, optionally substituted with one or more than one substituents independently selected among the group consisting of halogen, alkyl, halogenoalkyl, cycloalkyl, alkoxy, halogenalkoxy and hydroxy;

aryl, optionally substituted with one or more than one substituents independently selected among the group consisting of halogen, alkyl, halogenoalkyl, alkoxy, aryloxy, halogenoalkoxy, alkylthio, hydroxy, nitro, alkylsulfinyl, alkylsulfonyl, sulfamoyl, N-alkylsulfamoyl, N,N-di-alkylsulfamoyl, alkylsulfonamido and alkylsulfon-N-alkylamido; or

an aromatic or non-aromatic, mono- or bicyclic, optionally benzoannellated, heterocyclic group having 1, 2 or 3, heteroatoms independently selected from N, O and S and optionally being substituted with one or more than one substituents independently selected among the group consisting of halogen, alkyl, halogenoalkyl, alkoxy, aryloxy, halogenoalkoxy, alkylthio, hydroxy, nitro, alkylsulfinyl, alkylsulfonyl, sulfamoyl, N-alkylsulfamoyl, N,N-di-alkylsulfamoyl, alkylsulfonamido and alkylsulfon-N-alkylamido;

the third of radicals R1, R2, R3 represents

H, alkyl, halogenoalkyl, hydroxyalkyl, -CHO, -COOH, halogen, cyano, alkylsulfonyl, sulfamoyl or B-Y;

wherein

B represents alkylene or alkenylene, optionally substituted with hydroxy or alkoxy;

Y represents -COOH, SO_3H, $OPO(OH)_2$, $OP(OH)_2$, -CHO or tetrazolyl; or

the second and the third of radicals R1, R2, R3 represent,

together with the atom they are attached to, saturated or unsaturated cycloalkyl;

R4-R11, which may be the same or different, represent

hydrogen, alkyl, hydroxyalkyl, alkoxyalkyl, hydroxy, COOH or acyloxy, where vicinal radicals may also represent bonds or geminal radicals, together with the C atom they are attached to, may also represent carbonyl or cycloalkyl;

R12 represents

hydrogen, alkyl or phenyl,

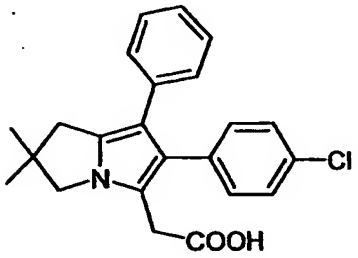
and optical isomers, physiologically acceptable salts and derivatives thereof,

for preparing a pharmaceutical composition for inhibiting gastric proton pump.

2. The use according to Claim 1, wherein the first and the second of radicals R1, R2, R3 independently represent an optionally substituted aryl or aromatic heterocyclic residue.

3. The use according to Claim 1 or Claim 2, wherein the third of radicals R1, R2, R3 represents COOH or B-Y, wherein Y is COOH and B represents alkylene.

4. The use according to Claim 1 wherein said compound of formula (I) is [6-(4-chlorophenyl)-2,2-dimethyl-7-phenyl-2,3-dihydro-1H-pyrrolizine-5-yl]-acetic acid of the formula (Ia)



a physiologically acceptable salt or a physiologically hydrolysable ester thereof.

5. The use according to Claim 1, wherein the gastric proton pump is gastric H^+,K^+-ATPase.

6. The use according to Claim 1, for inhibiting gastric acid secretion.

7. The use of one or more than one compound of Formula (I) as defined in any one of claims 1 to 4 for preparing a pharmaceutical composition for reducing the sensitivity of gastric mucosa to topical injury in a mammal in need of such treatment.

8. The use according to Claim 7, wherein the topical injury is caused by irritants.

9. The use according to Claim 8, wherein the irritants are selected from the group consisting essentially of NSAIDs and COX-inhibitors.

10. The use of one or more than one compound of Formula (I) as defined in any one of claims 1 to 4 for preparing a pharmaceutical composition for treating or preventing gastric acid-related conditions.

11. The use according to Claim 10, for treating or preventing gastric mucosal lesions.

12. The use according to Claim 10, for treating or preventing erosive gastritis.

13. The use according to Claim 10, for treating or preventing non-erosive gastritis.

14. The use according to Claim 10, for treating or preventing gastric ulceration.

15. The use according to Claim 10, for treating or preventing Ulcus duodeni or Ulcus ventriculi.

16. The use according to any one of the preceeding Claims, including in addition to one or more than one compound of Formula (I) one or more members selected from the group consisting essentially of antibacterially active agents, further gastroprotective agents such as further proton inhibitors and H_2-receptor antagonists, antacid agents, alginates and prokinetic agents.

17. A combination of (i) one or more than one compound of Formula (I) as defined in any one of claims 1 to 4, with (ii) one or more than one ulcerogenic anti-inflammatory agent for use in therapy.

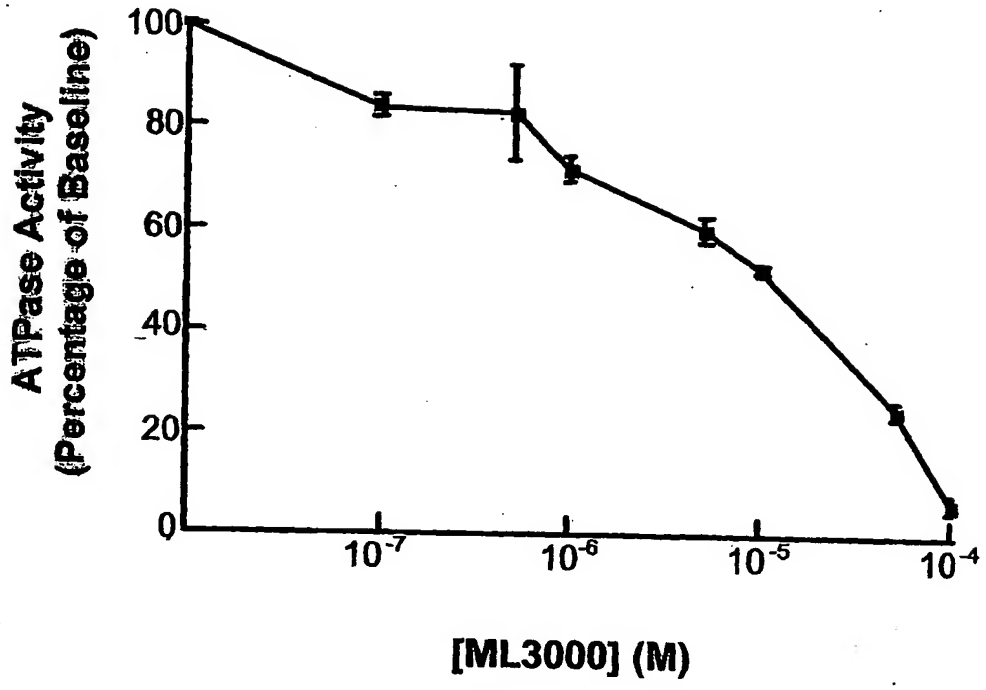
18. The combination of claim 17, wherein the ulcerogenic anti-inflammatory agent is selected from the group consisting of acetylsalicylic acid (ASA), sodium salicylate, acetaminophen, phenacetin, ibuprofen, ketoprofen, indomethacin, flurbiprofen, diclofenac, naproxen, piroxicam, tebufelone, etodolac, nabumetone, tenidap, alcofenac, antipyrine, amimopyrine, dipyrone, animopyrone, phenylbutazone, clofezone, oxyphenbutazone, prexazone, apazone, benzydamine, bucolome, cinchopen, clonixin, ditrazol, epirizole, fenoprofen, floctafeninl, flufenamic acid, glaphenine, indoprofen, meclofenamic acid, mefenamic acid, niflumic acid, salidifamides, sulindac, suprofen, tolmetin, nabumetone, tiaramide, proquazone, bufexamac, flumizole, tinoridine,

timegadine, dapsone, diflunisal, benorylate, fosfosal, fenclofenac, etodolac, fentiazac, tilomisole, carprofen, fenbufen, oxaprozin, tiaprofenic acid, pirprofen, feprazone, piroxicam, sudoxicam, isoxicam, celecoxib, tenoxicam.

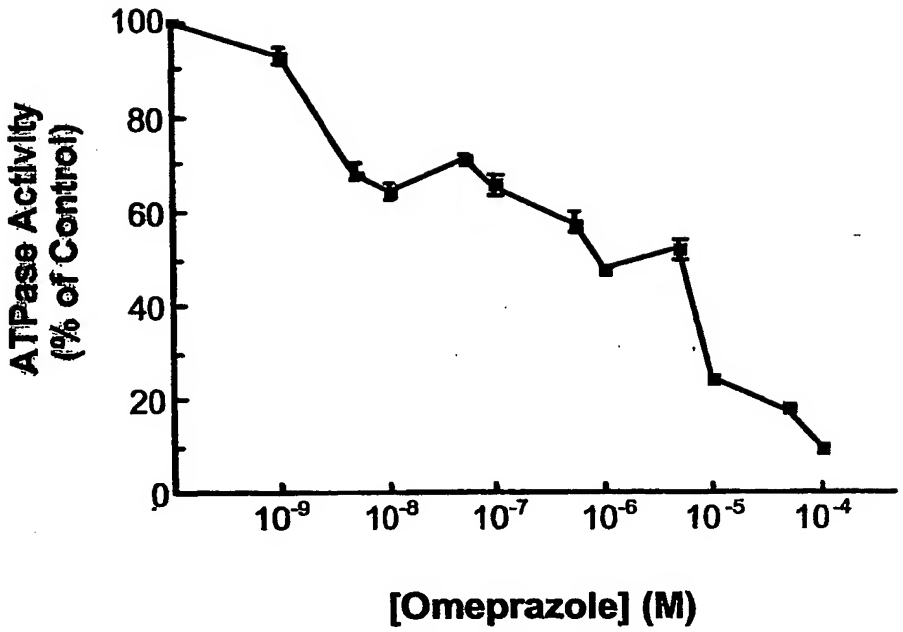
19. A pharmaceutical composition comprising (i) one or more than one compound of Formula (I) as defined in any one of claims 1 to 4, (ii) one or more than one ulcerogenic anti-inflammatory agent for use in therapy, and optionally (iii) an pharmaceutically acceptable carrier.

20. The composition of claim 19, wherein the ulcerogenic anti-inflammatory agent is selected from the group consisting of acetylsalicylic acid (ASA), sodium salicylate, acetaminophen, phenacetin, ibuprofen, ketoprofen, indomethacin, flurbiprofen, diclofenac, naproxen, piroxicam, tebufelone, etodolac, nabumetone, tenidap, alcofenac, antipyrine, amimopyrine, dipyrone, animopyrone, phenylbutazone, clofezone, oxyphenbutazone, prexazone, apazone, benzydamine, bucolome, cinchopen, clonixin, ditrazol, epirizole, fenoprofen, floctafeninl, flufenamic acid, glaphenine, indoprofen, meclofenamic acid, mefenamic acid, niflumic acid, salidifamides, sulindac, suprofen, tolmetin, nabumetone, tiaramide, proquazone, bufexamac, flumizole, tinoridine, timegadine, dapsone, diflunisal, benorylate, fosfosal, fenclofenac, etodolac, fentiazac, tilomisole, carprofen, fenbufen, oxaprozin, tiaprofenic acid, pirprofen, feprazone, piroxicam, sudoxicam, isoxicam, celecoxib, tenoxicam.

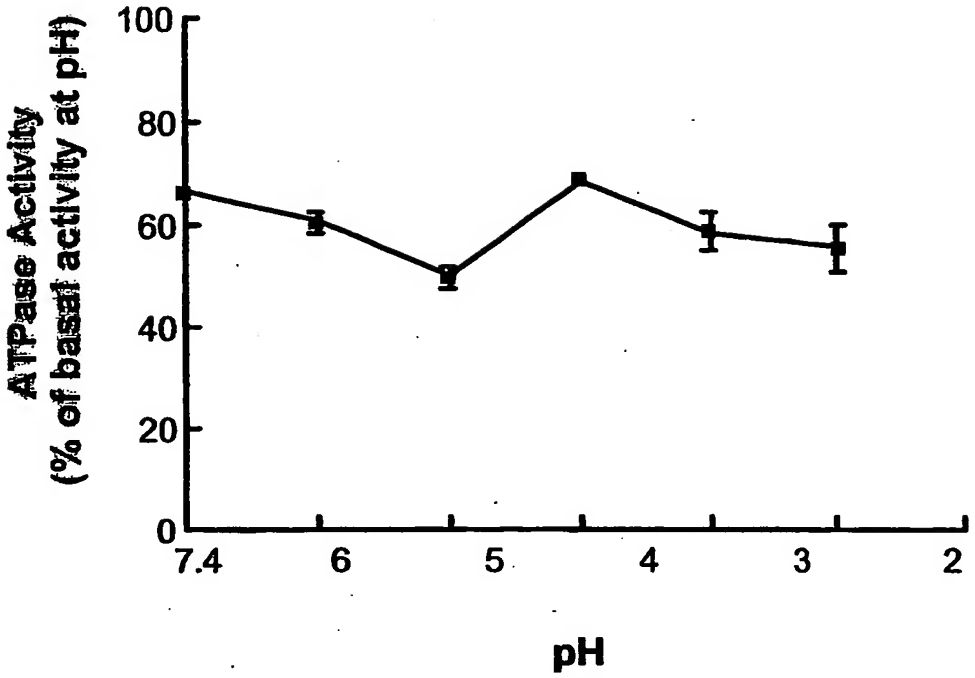
Figur 1


ATPase Activity
(Percentage of Baseline)
100
80
60
40
20
0
10^{-7}
10^{-6}
10^{-5}
10^{-4}
[ML3000] (M)

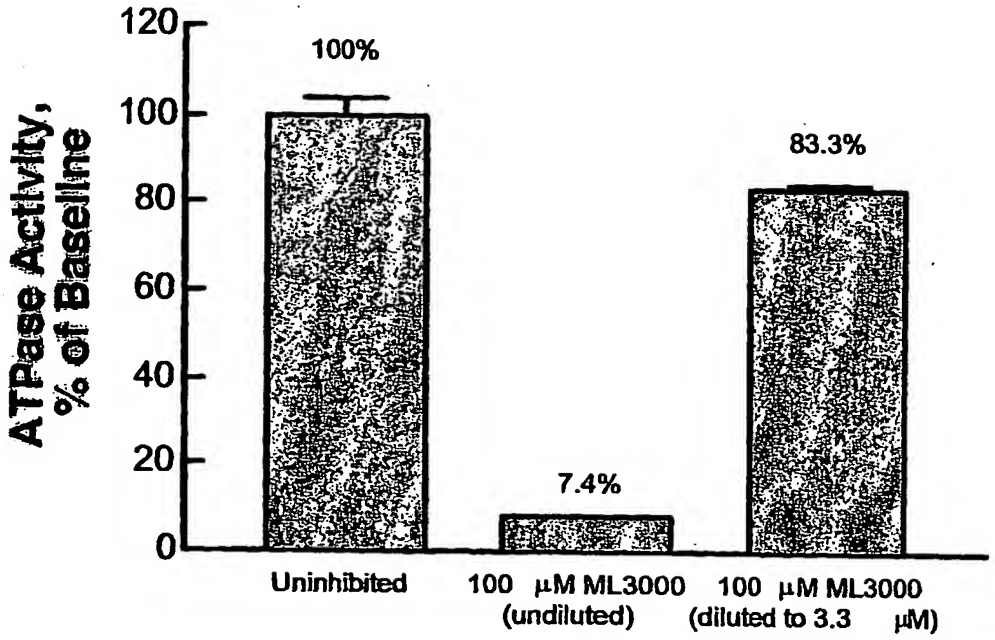
Figur 2


ATPase Activity
(% of Control)
100
80
60
40
20
0
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
[Omeprazole] (M)

Figur 3

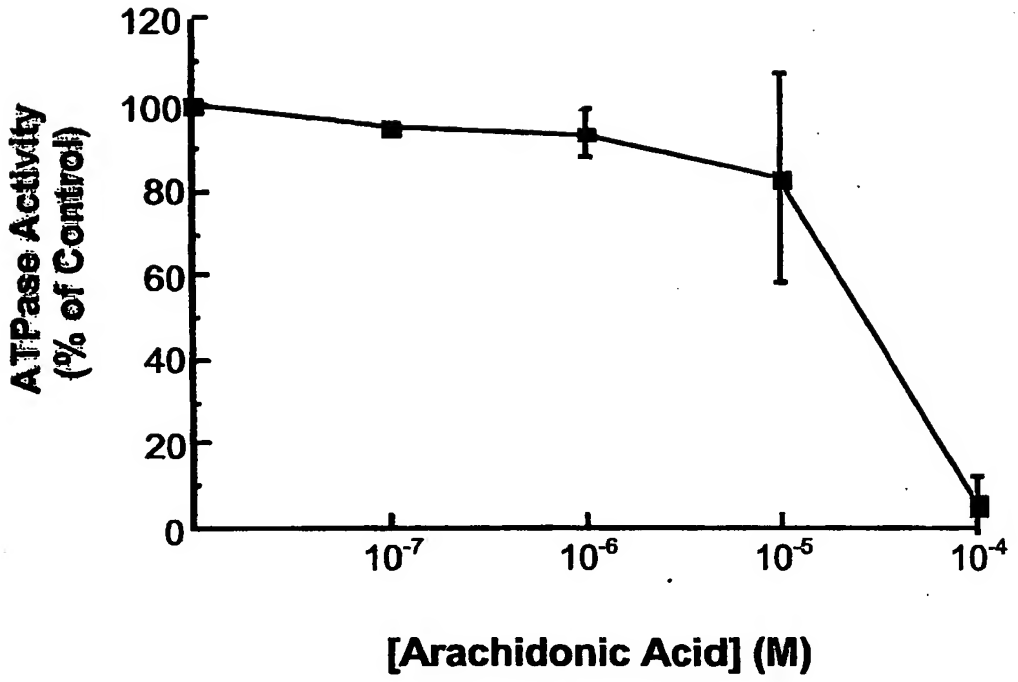

ATPase Activity
(% of basal activity at pH)
100
80
60
40
20
0
7.4
6
5
4
3
2
pH

Figur 4

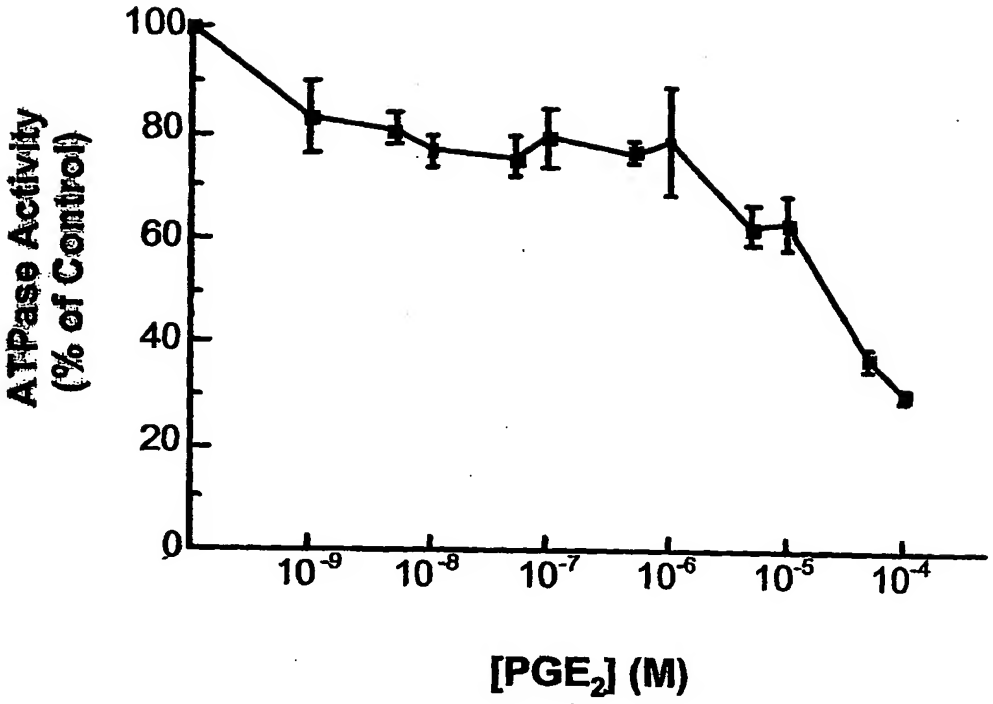

ATPase Activity, % of Baseline
120
100
80
60
40
20
0
100%
7.4%
83.3%
Uninhibited
100 µM ML3000 (undiluted)
100 µM ML3000 (diluted to 3.3 µM)

5/20

Figur 5

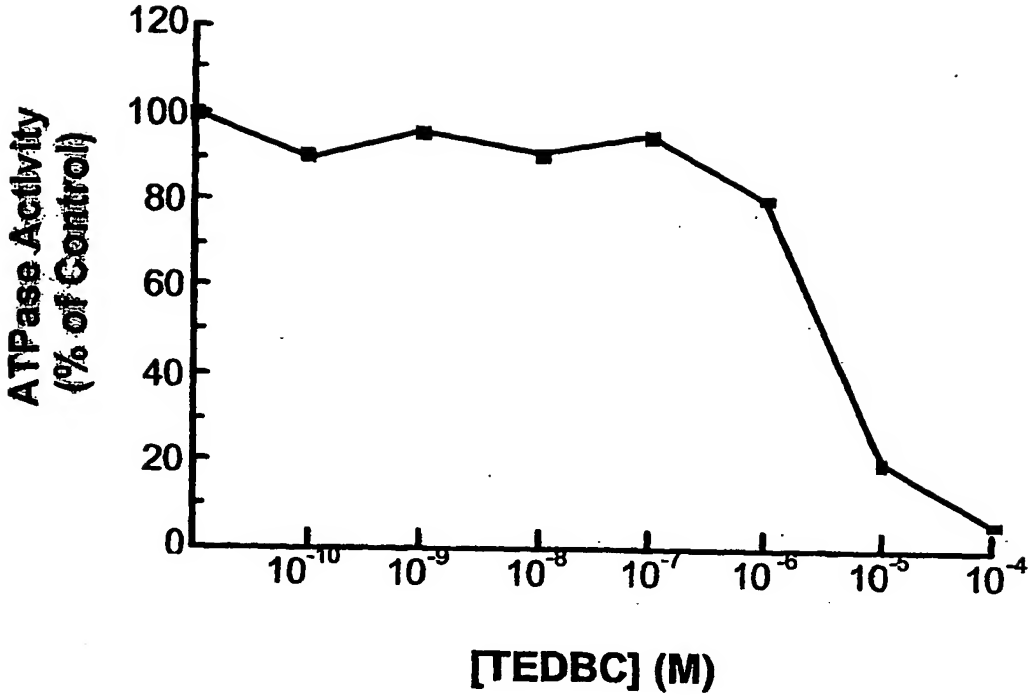

ATPase Activity
(% of Control)
120
100
80
60
40
20
0
10-7
10-6
10-5
10-4
[Arachidonic Acid] (M)

Figur 6


ATPase Activity
(% of Control)
100
80
60
40
20
0
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
[PGE_2] (M)

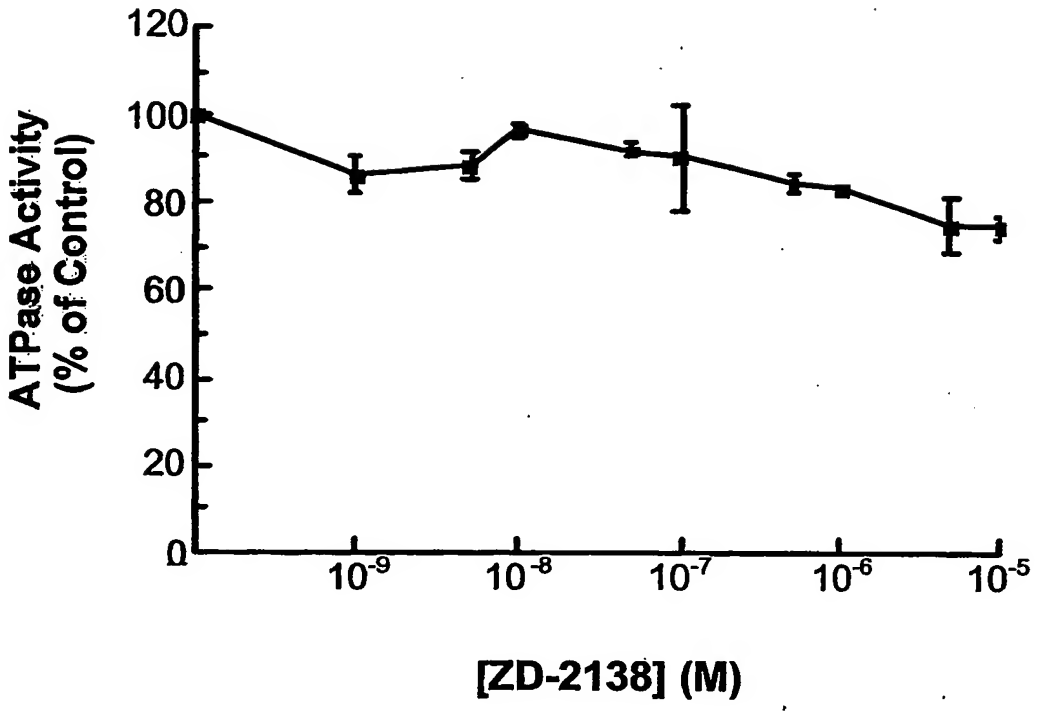
7/20

Figur 7

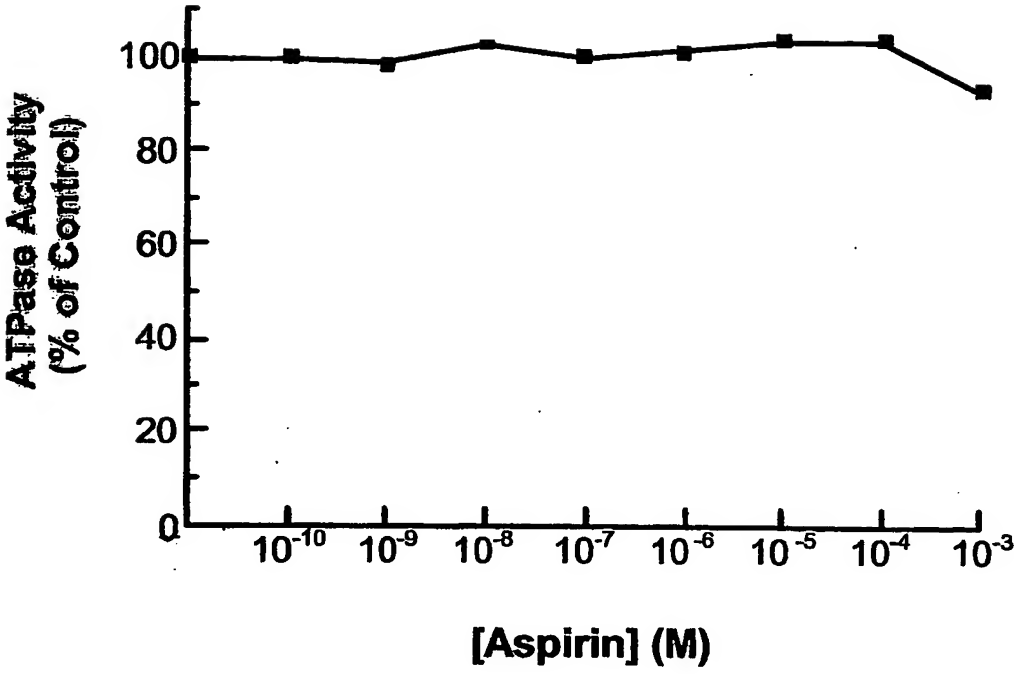

ATPase Activity
(% of Control)
120
100
80
60
40
20
0
10-10
10-9
10-8
10-7
10-6
10-5
10-4
[TEDBC] (M)

8/20

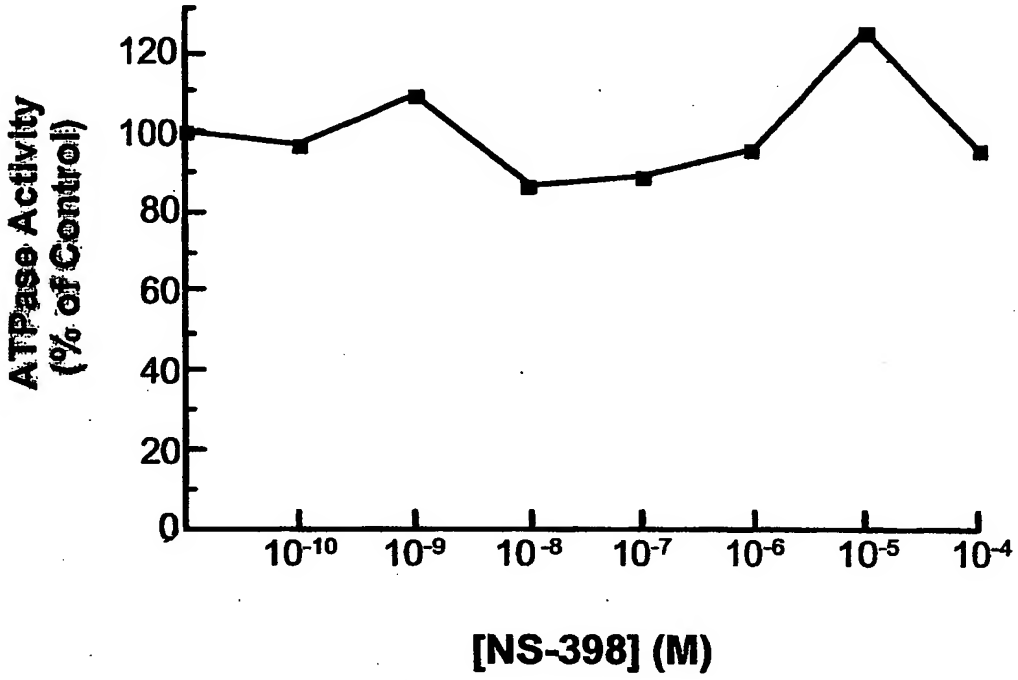
Figur 8


ATPase Activity
(% of Control)
120
100
80
60
40
20
0
10-9
10-8
10-7
10-6
10-5
[ZD-2138] (M)

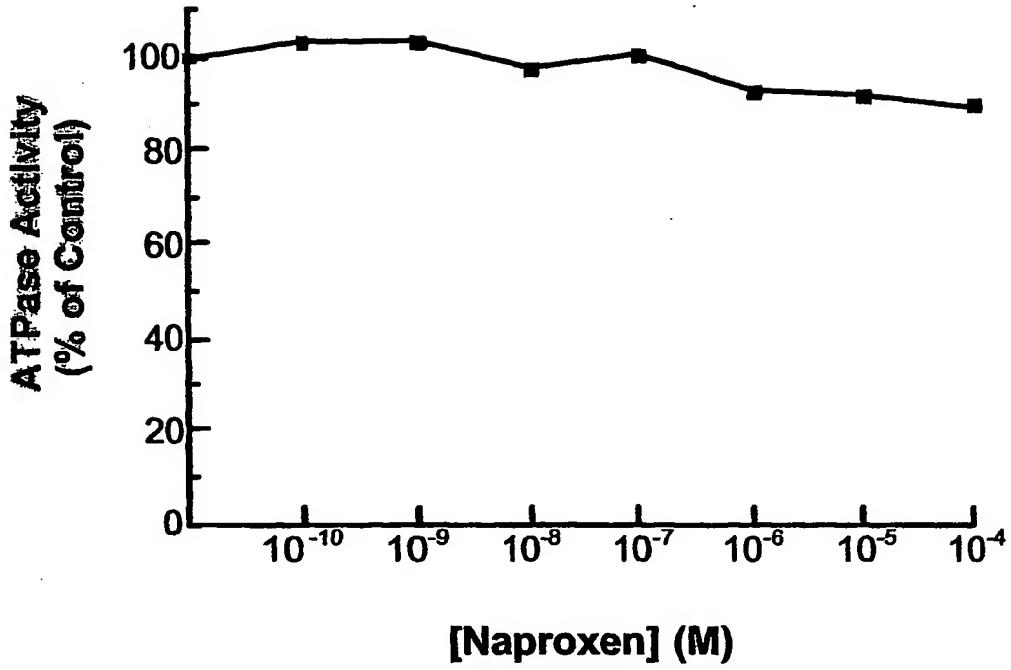
Figur 9


ATPase Activity
(% of Control)
100
80
60
40
20
0
10^{-10}
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
10^{-3}
[Aspirin] (M)

Figur 10

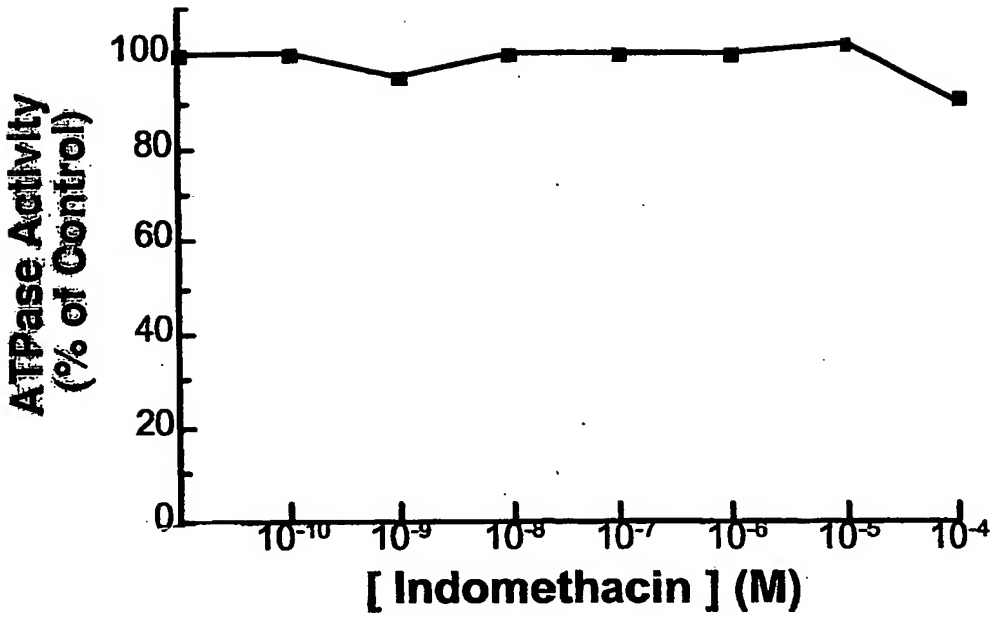

ATPase Activity
(% of Control)
120
100
80
60
40
20
0
10-10
10-9
10-8
10-7
10-6
10-5
10-4
[NS-398] (M)

Figur 11


ATPase Activity
(% of Control)
100
80
60
40
20
0
10^{-10}
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
[Naproxen] (M)

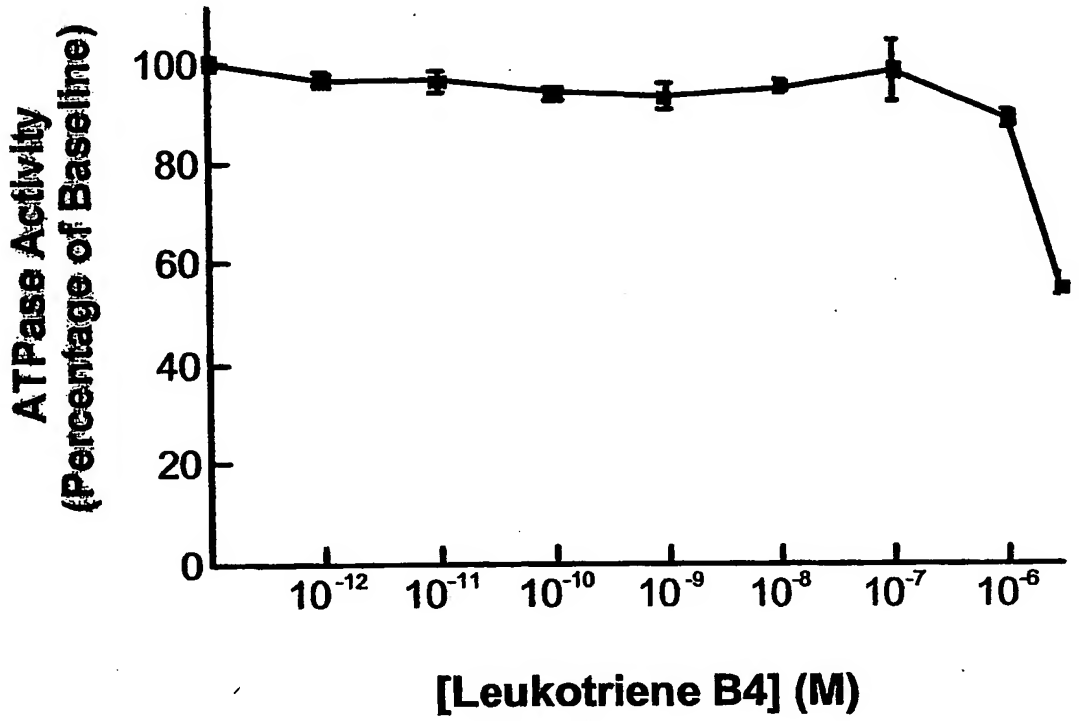
12/20

Figur 12



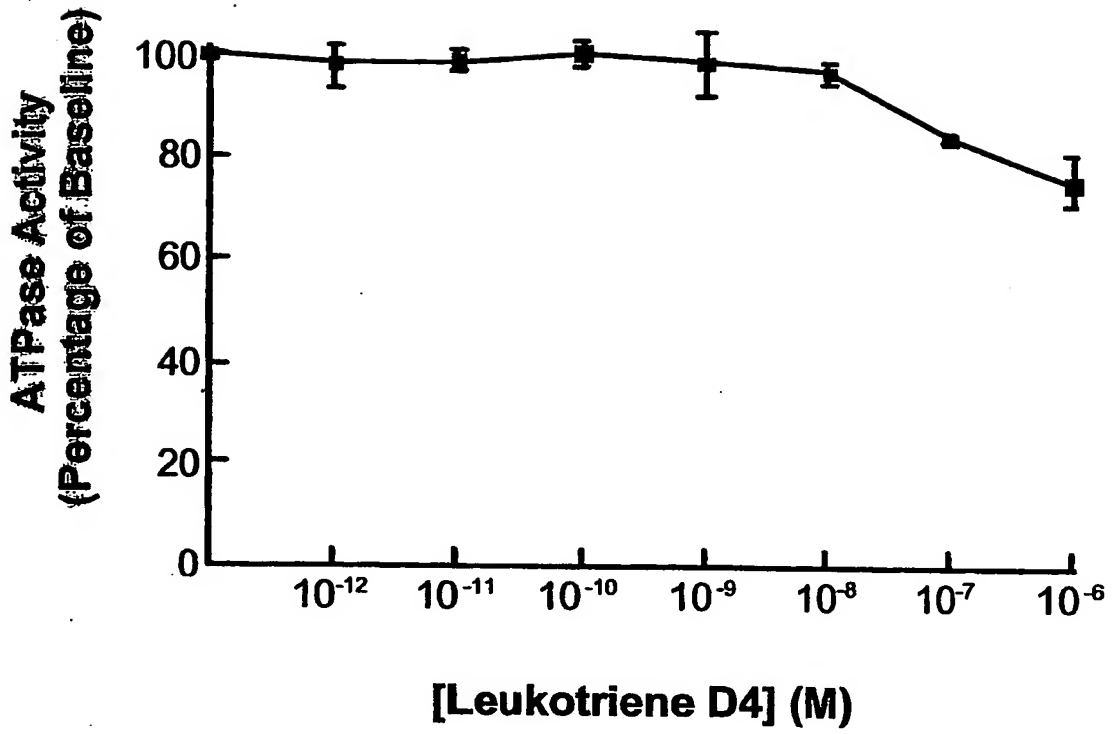
13/20

Figur 13

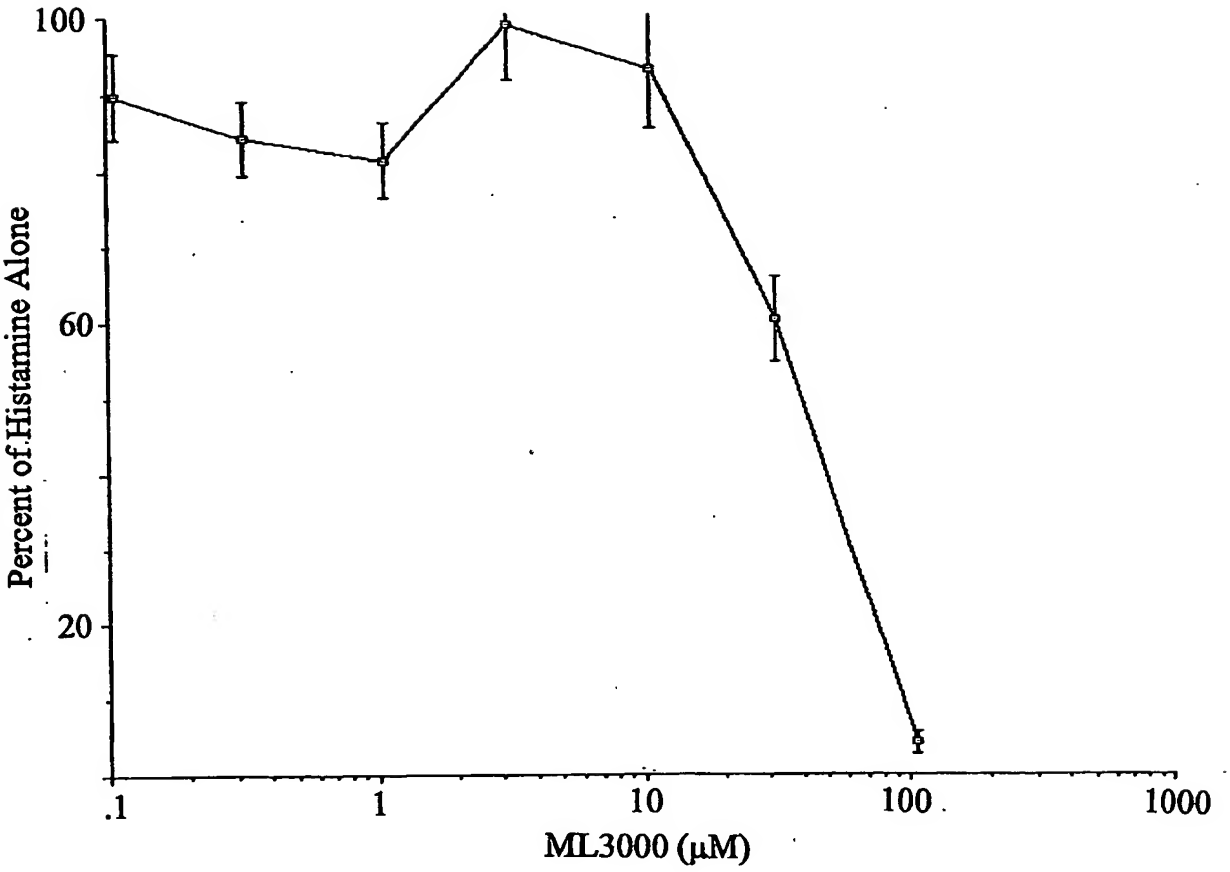


14/20

Figur 14

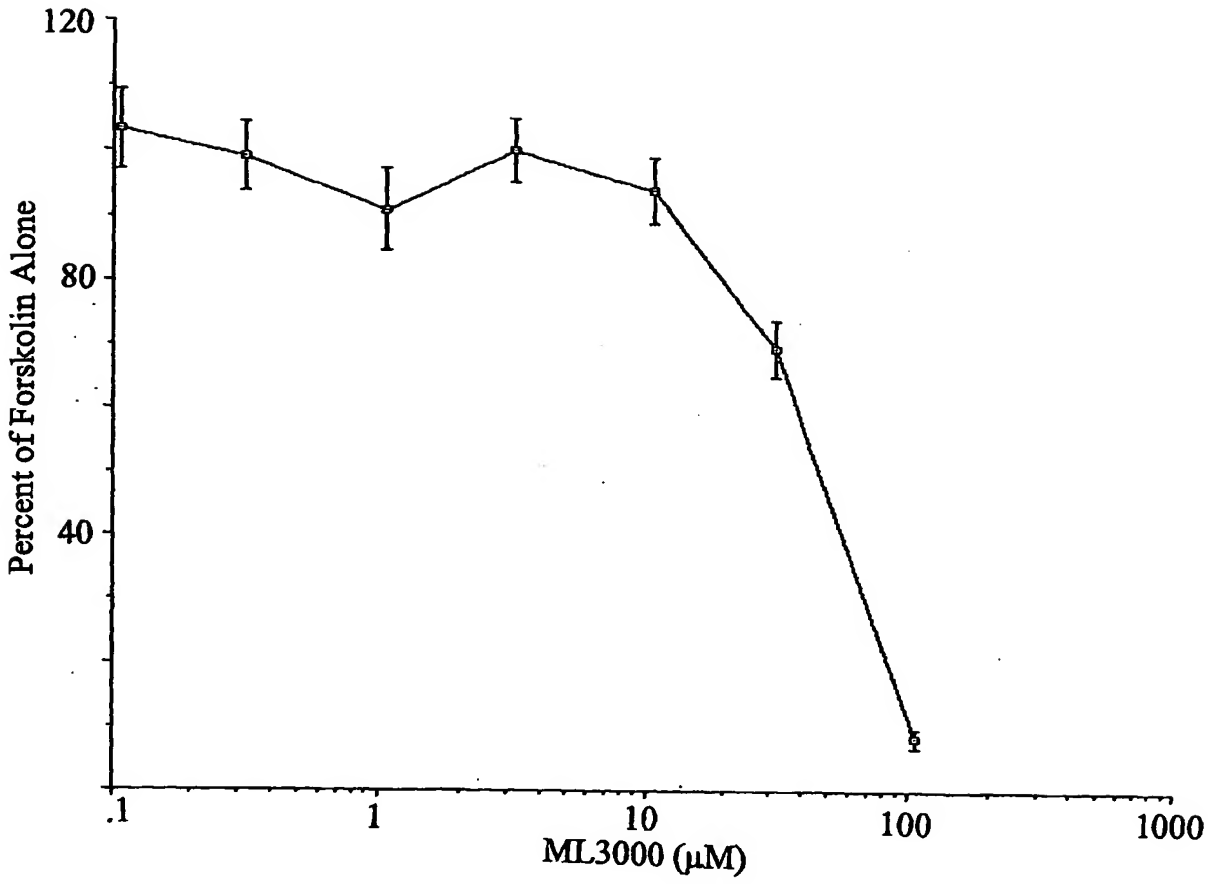


Figur 15

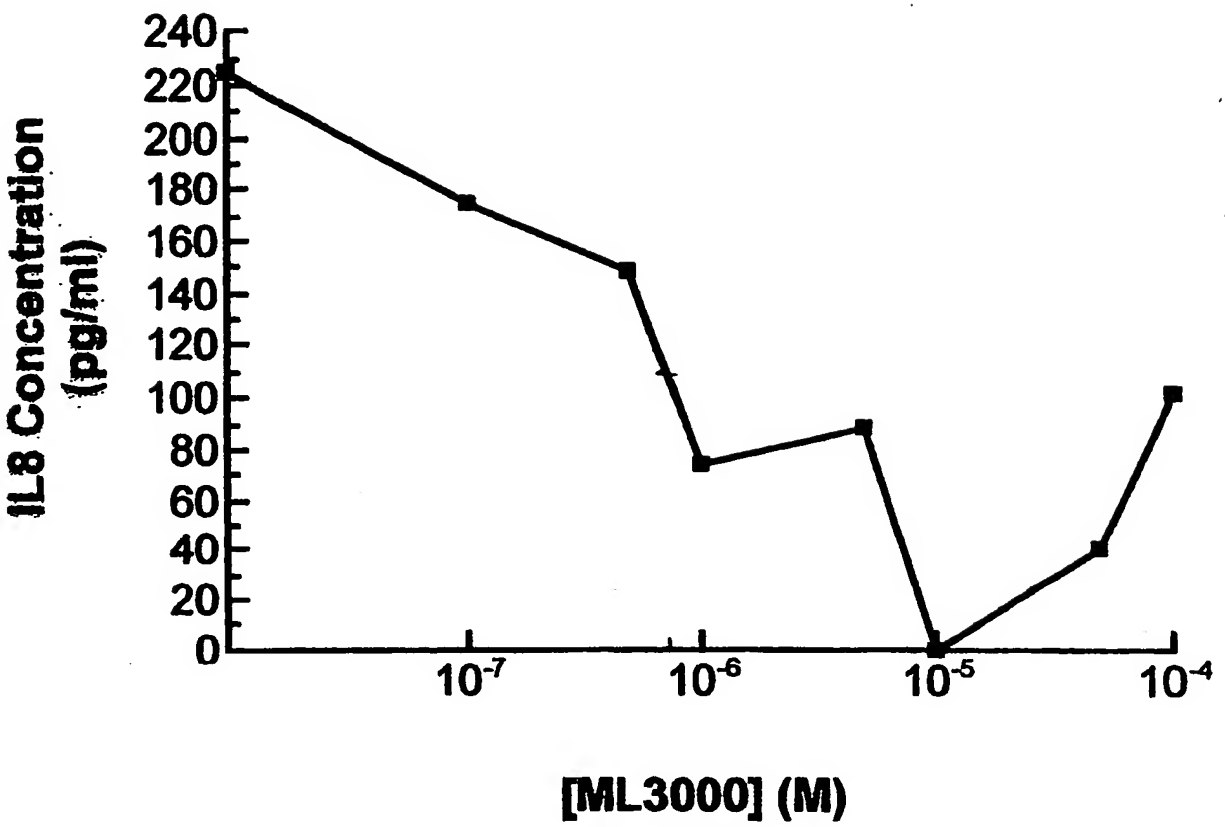


16/20

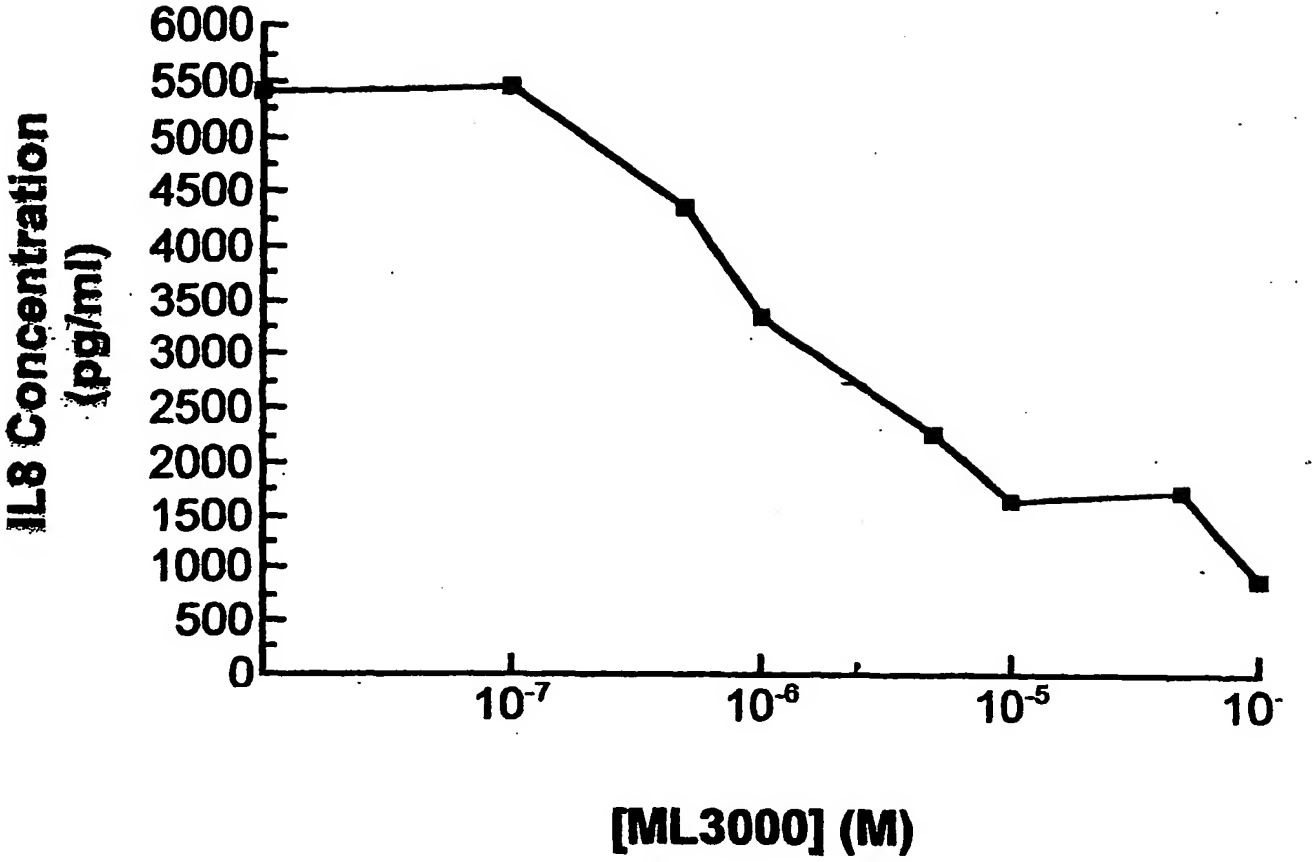
Figur 16



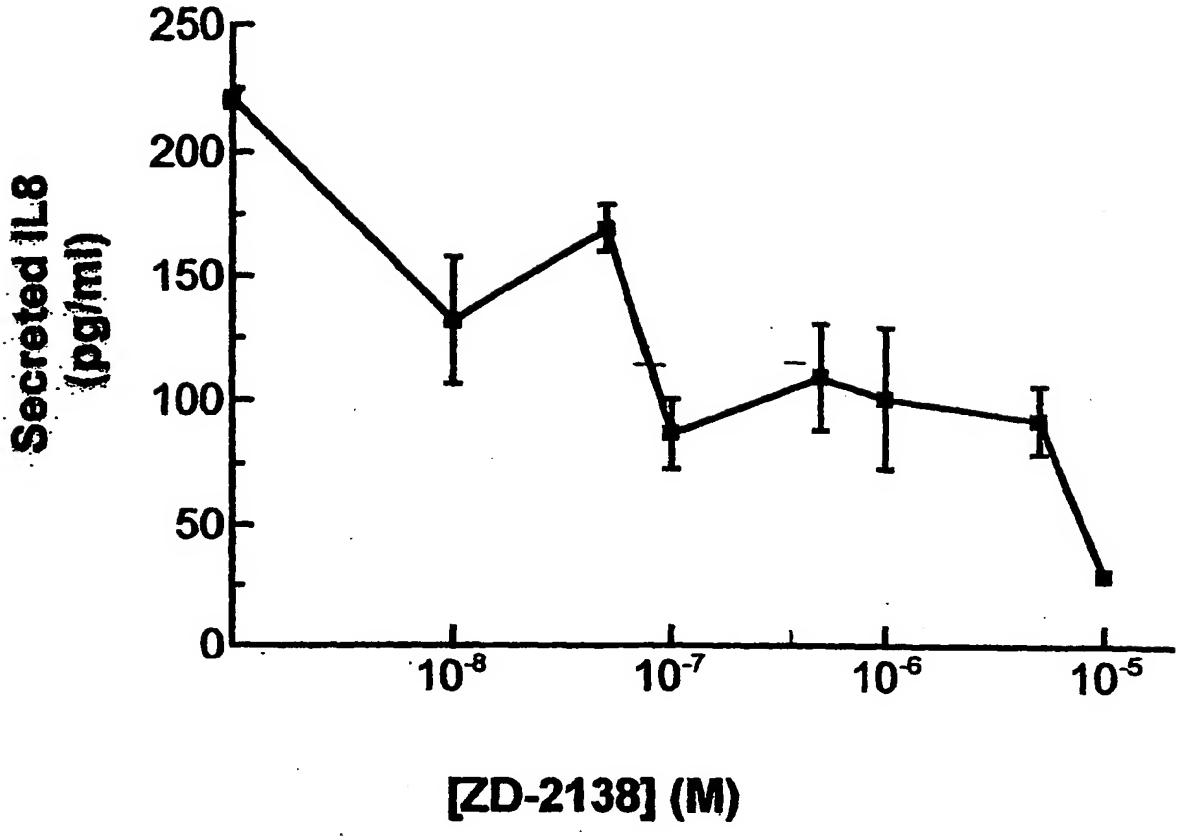
Figur 17


IL8 Concentration (pg/ml)
240
220
200
180
160
140
120
100
80
60
40
20
0
10^{-7}
10^{-6}
10^{-5}
10^{-4}
[ML3000] (M)

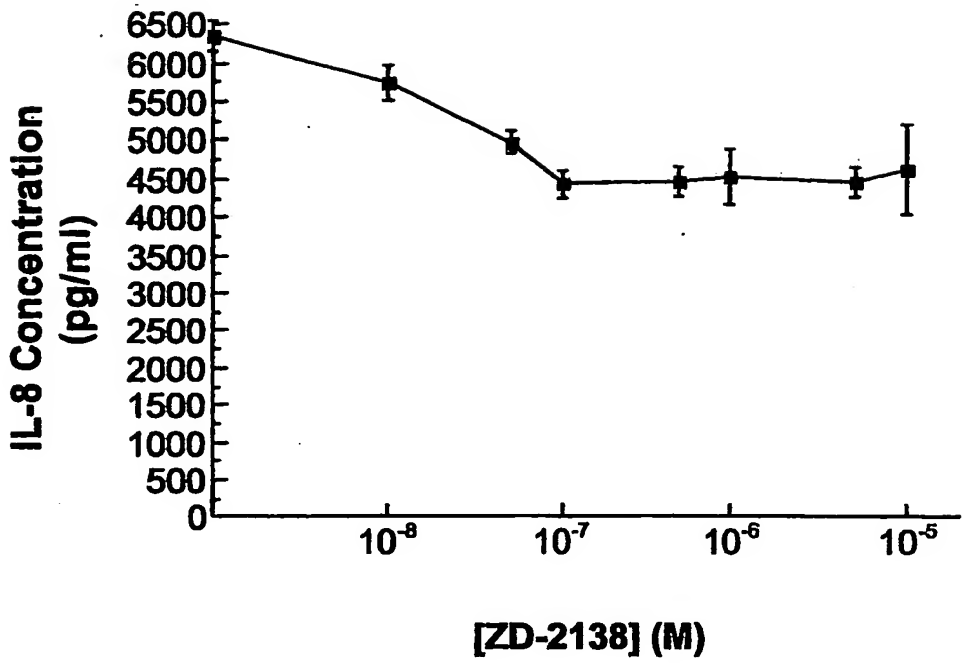
Figur 18


IL8 Concentration (pg/ml)
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
10-7
10-6
10-5
10-
[ML3000] (M)

Figur 19



Figur 20


IL-8 Concentration (pg/ml)
6500
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
10-8
10-7
10-6
10-5
[ZD-2138] (M)

INTERNATIONAL SEARCH REPORT

PCT/EP 03/05171

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/403 A61P1/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

MEDLINE, EMBASE, CHEM ABS Data, EPO-Internal, WPI Data, PAJ, BIOSIS, SCISEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WALLACE, J. L. ET AL: "ML 3000 reduces gastric prostaglandin synthesis without causing mucosal injury" EUROPEAN JOURNAL OF PHARMACOLOGY (1994), 271(2/3), 525-31, XP008008664 page 527, paragraph 2	1,2,4-16
X	WO 96 41626 A (SEARLE & CO) 27 December 1996 (1996-12-27) page 8, line 25 - line 36 claims 1,2,4 page 6, line 31 - line 36	1-15, 17-20

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 September 2003	19/09/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Bonzano, C

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LAUFER S ET AL: "Gastrointestinal tolerance of '2,2-dimethyl-6-(4-chlorophenyl-7-phenyl-2,3-dihydro-1H-pyrrolizine-5-yl!-acetic acid in the rat." ARZNEIMITTEL-FORSCHUNG, (1994 DEC) 44 (12) 1329-33., XP001108855 page 1329, column 1, line 1 -page 1330, column 1, line 13 page 1332, column 2, paragraph 3 - paragraph 5 tables 2,3	
A	TRIES, S. ET AL: "The pharmacological profile of ML3000: a new pyrrolizine derivative inhibiting the enzymes cyclo-oxygenase and 5-lipoxygenase" INFLAMMOPHARMACOLOGY (2001), 9(1-2), 113-124, XP008008717 page 122, line 1 -page 123, line 12 figure 11; table 2	
A	US 5 852 033 A (FERNANDEZ PUENTES JOSE LUIS ET AL) 22 December 1998 (1998-12-22) table 1	
A	WO 95 32970 A (MERCKLE GMBH ;LAUFER STEFAN (DE); STRIEGEL HANS GUENTHER (DE); DAN) 7 December 1995 (1995-12-07) page 2, line 6 - line 10 page 27; table 4	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 03/05171

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: -
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims 1-10,16 relate to therapeutic applications which are actually not well defined. The use of the definitions "inhibiting gastric proton pump, inhibing gastric secretion, reducing the sensitivity of mucosa to topical injury, gastric acid-related conditions" is considered to lead to a lack of clarity within the meaning of Article 6 PCT.
The lack of clarity is such as to render a meaningful complete search not fully possible.
Furthermore, present claims 1-3,5-20 relate to an extremely large number of possible compounds (compounds of formula I, claim 1; and the compounds listed in claim 18). Support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds claimed (compound ML3000, alone). In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible.
Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the compound mentioned in claim 4 alone, for treating the diseases specified in claims 11-15 with due regard to the general idea underlying the present invention.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

PCT/EP 03/05171

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9641626 A	27-12-1996	AU 6111796 A	09-01-1997
		CA 2224517 A1	27-12-1996
		EP 0833622 A1	08-04-1998
		JP 11507670 T	06-07-1999
		WO 9641626 A1	27-12-1996
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		DE 69616792 T2	20-06-2002
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		FI 964771 A	27-01-1997
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		US 5958943 A	28-09-1999